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DEVELOPMENT AND RESISTING POWER OF CLADOCERA EMBRYOS IN THE SOLUTIONS OF CERTAIN INORGANIC SALTS

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Zoologie et Anatomie.

Rozwój i odporność zarodków wioślarek w roztworach niektórych soli nieorganicznych. — Development and resisting power of Cladocera embryos in the solutions of certain inorganic salts.

Mémoire

de M. **MIROSŁAW RAMUŁT,**

présenté, dans la séance de 1 Décembre 1924, par M. M. Siedlecki m. t.

(Planche 8).

The present publication is in some connection with my earlier paper published in 1914 (26). In the Chapter VIII of that paper I paid special attention to the resisting power of Daphnia embryos in their early stages to the action of sodium chlorid and cane sugar solutions. Since that time have appeared the works of H. Fritzsche (6) and St. J. Przyłęcki (25,26), dealing with the inner osmotic pressure in various stages of development in Daphnia and Simocephalus. The work of Fritzsche deals with the osmotic pressure in the adult forms of Daphnia, these of Przyłęcki with the osmotic pressure in early embryonal stages. In those works a causal connection is proved between the osmotic pressure and some vital functions in Cladocera.

My experiments as described in the following work did not aim at the investigation of the inner osmotic pressure itself but at the investigation of some biological phenomena which are in a strict connection with this pressure. These are: 1) the influence of certain salt solutions upon the producing and laying of the parthenogenetic eggs in to the broodpouch, 2) the development of embryos of similar solutions, 3) the resisting power and acclimatisation of the Cladocera to these solutions in various embryonal stages.

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Material and methods.

The greater part of my experiments were carried out on *Daphnia pulex* de Geer, which proved to be the most convenient material: 1) because of its abundancy in the ponds in the neighbourhood of Cracow, 2) because it can be easily cultivated in aquariums. The most part of my experimental material consisting of *Daphnia pulex* had its origin in a long and narrow pond in Grzegórzki at Cracow, shallow and chocked up with Lemna; the bottom covered with a black mass of ooze and detritus. This material was cultivated in glass aquariums in the water which was a mixture of pond and tap water. — This species lived in aquariums and has shown stronger or weaker reproduction the whole winter. To some series of experiments were used (as in my former work) several other Cladocera species: *Simocephalus vetulus*, *Ceriodaphnia laticaudata*, *Scapholeberis mucronata*, *Camptocercus rectirostris*, *Pleuroxus aduncus*, *Polyphemus pediculus*. The cultivation of the above mentioned species, except the two first ones, was more difficult than the cultivation of the species *Daphnia pulex*, therefore the said experiments were made mostly on the fresh material brought from the ponds.

My experiments consisted mostly in the establishing of artificial cultures of various chemical compound into which I transferred the adult individuals resp. the embryos of *Daphnia pulex* or other similar species from their natural medium resp. from an aquarium with fresh water. These cultures contained either the pond water, (resp. aquarium water) or the tap water, or the distilled water, or the solutions of various mineral salts i. e. of the osmotically active, the electrolitic dissociation undergoing substances. — Only such salts were used in the solutions, which are present in the natural medium of the investigated species, and which are therefore of a biological importance for these species. These were: NaCl, KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$. The NaCl was often used in the solutions alone, the rest of the salts mostly in the compound solutions, as secondary components beside NaCl as the principal component. Of the compound solutions were used: 1) van't Hoff's solution with the proportion of the particular salts coresponding to the proportion of like salts in the sea water (100 NaCl + 2·1 KCl + 1·5 $CaCl_2$ [1])

[1]) the amount of $CaCl_2$ in the sea water is not constant, it decreases in certain seas till 1·0 molecular units (investigations of Loeb).

+ 3·8 $MgSO_4$ + 7·8 $MgCl_2$ of the molar units), 2) the solution which differed from the former by the lack of magnesium salts and which was almost identical with the Ringer-Locke solution. — Therefore in the description of my experiments I define this solution constantly as Ringer- Locke solution. — The van't Hoff- and modified Ringer- Locke solutions were used in different degrees of dilution, whereby the proportion of particular components remained unchanged. When in the same series of experiments sodium chlorid-, van't Hoff- and Ringersolutions were used, when the question was to compare their action upon the animals resp. eggs, they were used in isotonic concentrations. — The solutions of higher concentration $\left(\frac{n}{1}\right)$ were made with distilled water, but they were diluted only with tap water or pond water in order to obtain the concentration necessary for the experiments. The distilled water could not be used for dilution as it weakens and even kills the Daphnias as many experiments have proved it indisputably.

Neither was it indifferent whether the tap or the pond water was used as diluter. In the first place because there existed a remarkable difference in chemical composition between the tap water and the water from the ponds whence my experimental material was derived, secondly because the tap water is from a practical point of view completely bereft of nutritious substances, which are abundant in the pond water either in the diluted state or as the living microorganisms. In my experiments which lasted often several days or longer, the question of the good feeding of experimental animals was of great importance; thereupon depends the growth, resistibility, reproductive power and many other vital processes. — One can supply the tap water with an appropiate food but the chemical difference between tap- and pond water is not removed in this way and the tap water itself by the adding of food ceases to be chemically defined. — For these reasons, differing from some other authors (E. Hirsch), I used the solutions prepared with tap water only to such cultures in which the nutritious substances were to be excluded, or in which the feeding was of no importance on account of the short duration of the experiment; the distilled water was used only in the question of its specific action; in the majority of experiments the natural water taken either directly from the pond or from the aquarium,

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in which Daphnias had been cultivated, was used as the diluter of the concentrated solutions. — Of course a solution prepared in such a manner was in the chemical sense a mixture of natural components with the experimental salts; but as in my experiments the amount of the components introduced prevailed very strongly over the natural components of the pond water, in spite of the presence of the latter a quite exact study of the action of the former upon the animals was made attainable.

Every series of experiments was accompanied by the parallel test cultures, prepared in pure pondwater (without experimental salts) with the animals originating from the same natural culture as those used to the experiments.

The experimental cultures were kept in glass vessels. The shape of which was round, the size different, depending on the amount of the animals used to the experiments, but all vessels belonging to one series of experiments were of the equal size, with the equal quantity of the experimental fluid.

On an average one experimental animal came in for 10 cm^3 of the solution; this quantity of fluid proved to be sufficient for the existence and development of animals, on the condition of their being well fed. — All experiments were carried out in the usual room temperature, 15°—18° C. The glasses with the cultures were placed on the table at the window and in the day they were exposed to a clear though dispersed light.

The salts used in the solutions were warranted by manufactures as chemically pure (from the firms Merck and Kahlbaum).

In the experiments with distilled water I used in the beginning the common once distilled water, having however no certainty as to its absolut chemical purity, I went on using thrice distilled water, the action of which, though in a very slight degree, was different to the action of the but once distilled water. (For the third time it was distilled in Jena glass vessels).

Below I give the chemical composition of the pond water from Grzegórzki near Cracow, whence directly or indirectly the greater part of my experimental material had its origin.

Passing hardness 12·0 germ. degrees, permament 11·2 germ. degrees, total hardness 23·2 german degrees.

The dry remainder from 1 l. water dried in 110° C — 0·642 g.
„ „ „ in 180° C — 0·629 g.
„ „ after calcination 0·5217 g.

Compounds of calcinated residue:

	contained in 1 l. water	% calcinated residue
CaO	0·2004 g.	38·48
MgO	0·0245 „	4·69
$Fe_2O_3 + Al_2O_3$ (mostly Fe_2O_3)	0·0019 „	0·36
SiO_2	0·0165 „	3·16
SO_3	0.1540 „	29·52
CO_2 (volumetric.)	0·0094 „	1·80
NaCl + KCl (KCl : NaCl = about 1 : 50)	0·0884 „	16·94
Cl (not contained in KCl + NaCl)	0·0171 „	3·28
the summary contents of mineral components	0·5122 g.	98·23%.

This analysis was carried out by Mr. Edward Kurzyniec, the assistant of the I. Chemical Institute of the University in Cracow. For the ready and desinterested aid he bestowed on my work in this manner I consider it my duty to express my great intebtedness to him.

Concerning the chemical compound of the tap water in Cracow I got some data from the City Chemical Laboratory and from the State Institute for Food Research. The amounts of mineral components are varying in different months and years. The total hardness of the tap water according to the data of these two institutions amounted 5·8 – 8·2 german degrees, according to one analysis of the I. Chemical Institute of the University amounted to 9·5 german degrees.

The foregoing analysis of the pond water shows that from the mineral components of this water the calcium salts (sulphate and acid carbonate) are predominant, then follows the sodium chlorid, then magnesium salts, and the amount of potassium chlorid is but very insignificant. — All these components (the calcium salts included) are in too small a percentage to be able visibly to influence the experimental animals in the conditions in which my experiments were made. — E. g. the percentage of sodium chlorid in the pond water amounted 0·00884%. It was about one tenth of the minimum which evokes, a visible reaction in Daphnias, when

used alone in the experiment. The percentage of potassium chlorid in the same pond water is hardly one hundreth of the analogical minimum. The only chemical moment which might play a rôle in the use of pond water in the experimental solutions would be the antagonistic action of the bivalent calcium ions contained in the pond water in the greater pencentage then other components, to the univalent sodium- and potasium ions introduced to the experiments. The effect of this antagonistic action is however very slight in comparison with the effect of the experimental salt itself in regard to the much higher percentage of the latter.

The laying of the eggs in to the broodpouch and their formation in the presence of the salt solutions.

The influence of the salts contained in the experimental solution, if it attains a sufficient concentration, must be marked on the development of the parthenogenetic eggs of Daphnia, though these eggs are closed in the broodpouch of the adult individuals. In Daphnia, like in most other Cladocera species, the broodpouch is not hermetically closed. The entrance from the hind part is closed in the normal rest position of the abdomen by means of the first and simultaneously the longest of the elastic dorsal processes with the end turned forwards. This process however goes off from the dorsal wall of the shell at every stronger downwards or forwards directed movement of the abdomen, and the surrounding fluid can easily mingle with the fluid of the broodpouch. — In consequence of these movements the Dapnia embryos can leave the broodpouch having reached their fiual stage. By this way also some solid bodies can get into the broodpouch from outside. As a proof may serve the fact that among the material brought from the pond I found twice adult exemplars of the small sized species *Chydorus sphaericus* contained in the broodpouches of the living females of *Daphnia pulex*. (Fig. 1.) For this reason if an egg producing female of the species *Daphnia pulex* or any other species used in my experiments (except *Polyphemus pediculus*) was placed in the solution, in the shortest time the eggs resp. embryos, closed in the broodpouch, were under the direct action of the same solution.

I found in the literature concerning the physiology of Daphni-

des some mentions of the power in these animals to the partheno-genitic reproduction in the solutions of mineral salts. — In Paul Bert's experiments (1883) a new *Daphnia pulex* generation appears

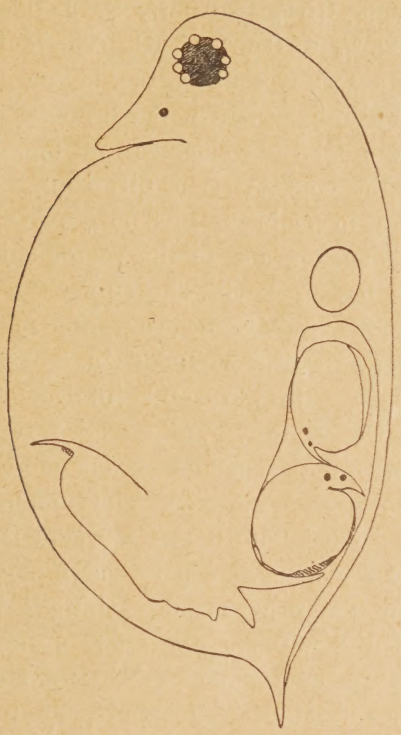

Fig. 1.

Daphnia pulex containing in the broodpouch 2 adult exemplars of *Chydorus sphaericus*.

in the mixture of sea water with fresh water, the concentration of which amounted to one third of the normal sea water concentration. This degree of concentration was attained gradually by the daily addition of a small quantity of sea water to the culture. The author does not mention however in which concentration the eggs were laid. The final concentration in his experiment coresponds to about $\frac{n}{6} - \frac{n}{5}$ sodium chlorid solution as regards the height of the osmotic pressure.

More data concerning this question are to be found in the E. Warren's work. (1900). The author gives the history of two

Daphnia magna stocks (families) bred in NaCl solutions. The individuals of these stocks were transferred in the course of experiments from the weaker to the stronger solutions: one stock from 0·2% NaCl through 0·5% to 0·55% NaCl, the other from 0·5% NaCl through 0·525% to 0·55% NaCl. The author in these experiments confirms a series of facts: the laying of the parthenogenetic eggs into the broodpouch in the presence of 0·2% and 0·5% NaCl solutions, the total development of the embryos and their further living in the concentration till 0·55% NaCl, the relaxing of the growth rate in embryos in 0·55% NaCl, and the impossibility of the development and of the reproduction in the concentration surpassing 0·55% NaCl. The author considers the last mentioned solution as the limitary concentration of NaCl — solution to which *Daphnia magna* can be acclimatised. — These results acquaint us in some measure with the ability of Daphnia embryos to develop in the NaCl solution. The author gives us however too scanty information for the obtaining of a more exact and a more grounded view upon this question. The number of experimented animals seems to be too limited (10 individuals in both stocks) to enable one to draw a general conclusion. A description is wanting as well as to how the experimental solutions were prepared and whether and how the animals were fed during the experiments. Besides this the test experiments (with the adult individuals) are wanting, which could allow us to appreciate the vitality and the reproductive power of the experimented material.

E. Hirsch in his work: „Untersuchungen über die biologische Wirkung einiger Salze" (1914) makes mention of the appearing of the new generation of Daphnia in the salt solutions. The experiments of H. had mainly in view the confirmation of the action of different salt solutions on the adult individuals. H. states that in some stocks bred in NaCl solutions $\left(\frac{n}{100}, \frac{n}{32}, \frac{n}{16}\right)$ after a time from 2 to 7 days the young appeared (the latter developed evidently from the eggs laid in the presence of these solutions). In the next higher concentration $\left(\frac{n}{8}\right)$ the egg production stoped. The intermediate concentrations between $\frac{n}{16}$ and $\frac{n}{8}$ NaCl are wanting in H.

experiments; thus the author accepts the concentration $\frac{n}{16}$ NaCl (about 0·36%) as the maximally tolerable (erträglich) for the parthenogenetic reproduction. This concentration may be regarded in his experiments as the maximum for capability of embryonal development in Daphnia.

Finally we find a mention in the H. Fritzsche's work (cited in the beginning) of the appearing of the new generation of *Dapnia magna* in the NaCl solutions of the concentration defined by $\Delta = -0{\cdot}11^{\circ}$ C and $-0{\cdot}20^{\circ}$ C (coresponding approximately to the $\frac{n}{30}$ and $\frac{n}{18}$ NaCl solutions, experiment 1.) and in the sea water solution defined by $\Delta = -0{\cdot}29^{\circ}$ C (a concentration isotonic with $\frac{n}{12}$ NaCl solution, experiment 5.); besides, when the author in another experiment did not transfer the adult Dapnias directly to the more concentrated sea-water solution, but began by very diluted solution and by a slow evaporation attained a higher concentration, he realised in this culture after several weeks the presence of three young in the solution of the concentration $\Delta = -0{\cdot}51^{\circ}$ C (the concentration isotonic with $\frac{n}{7}$ NaCl solution). However just as in the analogical experiment of P. Bert, the degree of the solution concentration in the time when the eggs were laid into the broodpouch is not known.

Most of my experiments were carried out in the NaCl-, Ringer- Locke-, and van't Hoff's solutions. NaCl-solutions were used in the concentrations $\frac{n}{50}$, $\frac{n}{40}$, $\frac{n}{30}$, $\frac{n}{24}$, $\frac{n}{20}$, $\frac{n}{15}$, $\frac{n}{12}$, $\frac{n}{10}$, $\frac{n}{8}$, $\frac{n}{7}$, Ringer- Locke- and van't Hoff's solutions in concentrations isotonic with $\frac{n}{40}$, $\frac{n}{30}$, $\frac{n}{24}$, $\frac{n}{20}$, $\frac{n}{15}$, $\frac{n}{12}$, $\frac{n}{10}$ NaCl solutions.

My experiments proved that in the low concentrations in *Daphnia pulex* and other similar species the parthenogenetic eggs are laid into the broodpouch without any difficulty as in the common freshwater whereas in the higher concentrations this function is often irregular. The eggs either are not laid at all, are retained in the ovaries and their yolk seems to undergo a resorbtion, or part of

them pass in to the broodpouch, another part remain in one or in both ovaries (Pl. I. fig. 1—5), or finally the mass of egg substance instead into the broodpouch is pressed into the abdominal part of body cavity which is possible only after the bursting of the subtile ovary wall. (Pl. I. fig. 4 5). The eggs which get into the broodpouch in such an irregular manner, are often abnormally small and deformed, (Pl. I. fig. 6), their surface is uneven owing to the extruding yolk-corns (Pl. I. fig. 7) and soon degenerate and desintegrate. Such eggs were never observed to develop. — This irregular laying of eggs was to be seen in some cases already in $\frac{n}{20}$ NaCl- and in the equally concentrated Ringer- and van't Hoff's solution, and the higher the concentrations the more irregular was this process. In the $\frac{n}{10}$ NaCl solution the eggs, if they were laid at all, were laid only partially and irregularly. — The adult animals, in which the laying of the eggs was disturbed in such a manner, continued to live apparently quite normally in the same experimental solution. — The irregular laying of the eggs I observed too in experiments with the species *Simocephalus vetulus*, *Ceriodaphnia* and *Pleuroxus aduneus* in the concentrations $\frac{n}{15}$ and $\frac{n}{12}$ NaCl.

Records of the experiments.

1) A series consisting of 7 cultures with *Daphnia pulex* = three cultures in $\frac{n}{10}$ NaCl-solution and isotonic Ringer- and van't Hoffsolution, three others in $\frac{n}{20}$ NaCl-solution and isotonic with it Ringer- and van't Hoffsolution, and one testculture in water from aquarium; the animals taken from the aquarium, all in a state of intensive parthenogenetic reproduction. — Each culture containing 16 adult females, 6 of which with mature eggs in the ovaries, 4 with eggs in first development stages in the broodpouch, and with immature eggs in the ovaries, and 6 with embryos in early stage after the bursting of the eggmembrane and with the immature eggs in the ovaries.

The course of the experiment was as follows:

Table I.

	$\frac{n}{10}$ NaCl	isotonic Ringer-solution	isotonic van't Hoff solution	$\frac{n}{20}$ NaCl	isotonic Ringer-solution	isotonic van't Hoff solution	pond water
after 12 hours eggs laid in	1 Dapnia	1	3	2	—	—	2
condition of adult animals	normal	normal	normal	normal	normal	normal	normal
after 36 hours eggs laid in	1	2	3	4	3	3	3
condition of adult animals	4 slightly weakened, 8 much weakened, 4 dead	2 weakened, 13 much weakened, 1 dead	6 weakened, 7 much weakened, 3 dead	10 normal, 3 weakened, 3 dead	1 weakened, 15 normal	15 normal, 1 weakened	all normal
after 60 hours condition of adult animals	all dead	all dead	all dead	weakened 8 dead	all weakened	all weakened	all normal

As regards the development of the eggs in this series, it was various, depending upon the concentration: the eggs laid in $\frac{n}{10}$ NaCl and isotonic Ringer- and van't Hoff's solution did not show any development, among the eggs laid in $\frac{n}{20}$ NaCl- and isotonic Ringer- and van't Hoff's solution some stopped at different stages of development, some attained the stage, in which they were able to lead an independent life.

The results of this series seem to show: 1) that the laying of the eggs meets with some difficulties in higher concentrations, 2) that the disturbing of this function is in some measure depending upon the weakening of the adult females, 3) that the Ringer- and van't Hoff's solutions act less noxiously then the equally concentrated NaCl-sotution.

2) Similar results were obtained in another series consisting of 9 cultures: $\frac{n}{10}$ NaCl solution, isotonic Ringer- and van't Hoffs solutions, $\frac{n}{20}$ NaCl-solution, isotonic Ringer- and van't Hoff's solutions, $\frac{n}{30}$ NaCl-solution, isotonic Ringer- and van't Hoff's solutions. The animals were of the same origin as in series 1). Every solution contained 12 individuals.

In this series the eggs were laid only by some individuals in NaCl- as well as in Ringer- and in van't Hoff's solutions; in NaCl-solutions however only in the concentrations $= \frac{n}{20}$ and $\frac{n}{30}$. On account of the fact that in $\frac{n}{10}$ NaCl no laying of eggs took place this experiment might indicate that the chemical composition of the medium has a certain though slight influence on that function. This influence, if existing at all, is much slighter than the influence of the chemical composition on the embryonal develepment which took place in this series evidently better in Ringer-solution than in isotonic NaCl-solutions, and better in van't Hoff- than in isotonic Ringer-solutions.

3) A series of cultures with another species of the same family, *Ceriodophnia laticaudata*, showed different results as regards the

Table II.

	$\frac{n}{12}$ NaCl	isotonic van't Hoff solution	$\frac{n}{15}$ NaCl	isotonic van't Hoff solution	$\frac{n}{20}$ NaCl	isotonic van't Hoff solution	pond water
after 6 hours eggs laid in	—	—	2	2	2	2	--
condition of adult animals	normal	normal	normal	normal	normal	normal	normal
after 24 hours eggs laid in	1 (irregul.)	—	2	3	2	3	7
condition of adult animals	normal	normal	normal	normal	normal	normal	normal
after 48 hours eggs laid in	1	—	2	3	2	7	12
condition of adult animals	10 weakened 5 dead	4 much weakened 11 dead	10 normal 5 weakened	7 normal 4 weakened 4 dead	9 normal 6 without pulsation	normal	normal

difference in action between sodium chlorid- and the compound solutions. — This series consisted of 7 cultures: 1 test culture with pondwater, 3 cultures in $\frac{n}{12}$, $\frac{n}{15}$ and $\frac{n}{20}$ NaCl-solutions, and 3 cultures in the van't Hoff's solutions isotonic with the former three. Every culture contained 15 individuals. From these 15 animals in every single culture were 7 animals with mature eggs in the ovaries, 3 with young freshly laid eggs in the broodpouch, and 5 with young embryos, still closed in the larval membranes.

No more eggs were laid in this series in the following days. Concerning the vitality of the adult, they lived longer in $\frac{n}{12}$ and $\frac{n}{15}$ NaCl solutions, than in isotonic van't Hoff's solutions. — The results of this series seem to show, that the noxious influence of van't Hoff's solution, both on the laying of the eggs and on the vitality of the adult, is weaker than the influence of the isotonic NaCl solution, but only in weaker concentration $\left(\frac{n}{20}\right)$; if more highly concentrated $\left(\frac{n}{12}\right)$ the NaCl-solution proved to be less noxious than the isotonic van't Hoff's solution (s. Table II).

4) One series of experiments was carried out on the species *Pleuroxus aduncus*, which as a Chydorid, both in the morphological and biological regard differs much from the species of the Daphnidae family.

The series consisted of 4 cultures: one test culture and 3 cultures in NaCl-solutions $\left(\frac{n}{20}, \frac{n}{15} \text{ and } \frac{n}{12}\right)$. Each of these cultures contained 8 animals. From there 8 animals in every culture were 4 with mature eggs in the ovaries and 4 with young embryos in the broodpouch closed in the larval membranes. The animals were taken from a small aquarium supplied with pond water in which Pleuroxus had been bred for two months.

The course of this series was following (s. Table III):

The adult individuals lived: in $\frac{n}{12}$ NaCl 3—5½ days, in $\frac{n}{15}$ NaCl 4—9 days, in $\frac{n}{20}$ NaCl some of Pleuroxus were observed

Table III.

	$\frac{n}{12}$ NaCl	$\frac{n}{15}$ NaCl	$\frac{n}{20}$ NaCl	pond water
after 24 hours eggs laid in	2 (irregular)	1	—	1
condition of adult animals	all normal	all normal	all normal	all normal
after 48 hours eggs laid in	2	2	2	3
condition of adult animals	all normal	all normal	all normal	all normal
after 72 hours eggs laid in	2	2	3	3
condition of adult animals	1 normal 4 weakened 3 dead	7 normal or slightly weakened 3 dead	all normal	all normal

alive still on the 10-th day of the experiment. This species in this series of experiments proved to have a stronger vitality than *Daphnia pulex* and *Ceriodapnia laticaudata* in analogical conditions. — The graduation of the noxious influence of the sodium chlorid solution was quite parallel to its concentration. — Although in the two first days the animals were apparently normal in $\frac{n}{12}$ NaCl solution, the eggs were laid irregularly and with difficulty, as though besides the vitality of the adult some other factors exercised an influence upon this function. — Occasionally I cite here the observations on the egg development in this series. The eggs laid in $\frac{n}{12}$ NaCl solution remained without any development, those laid in $\frac{n}{15}$ NaCl passed through their initial development stages inside the egg membrane, the eggs laid in $\frac{n}{20}$ NaCl passed also through their initial development stages and one only attained the stage characterised by primitive extremities. — The eggs laid in the test culture (pond water) passed through their development quite normally

(the total normal development till the moment of leaving the egg membrane lasted 5—6 days).

On the above cultures some observations were made concerning the further production of eggs, when the first brood had attained its final stage of development. In the test culture this production took place regularly: on the same or on the following day, when the embryos of the first brood hat left their broodpouches, new eggs passed into them. In the individuals living in the NaCl-solutions, the eggs of the first brood in the analogical moment remained in the ovaries. In order to evoke some change I transferred 2 animals with the maturing eggs in the ovaries from $\frac{n}{20}$ NaCl into the pond water. In both the eggs were laid in one after 56 hours, in another after 96 hours from the moment when the preceding brood had left the broodpouch. — This considerable retardation took place though the adult animals seemed to be in a normal condition.

As the experiments hitherto described gave no reliable account as to the question, whether on the retention of the mature eggs in the ovaries directly influences the higher osmotic pressure from outwards or rather the inner condition of the adult animals, I used in one series of cultures other salt, more chemically noxious than NaCl. This salt was KCl, which when used in equal concentrations, as NaCl in previous experiments $\left(\frac{n}{10}, \frac{n}{12}, \frac{n}{15}, \frac{n}{20}\right)$, kills Cladocera in a short time therefore can be used only in much lower concentrations.

5) The series consisted of 2 cultures: in $\frac{n}{60}$ KCl- and in $\frac{n}{120}$ KCl solution. 6 *Daphnia pulex* with mature parthenogenetic eggs in the ovaries were put into $\frac{n}{60}$ KCl: after 24 hours abode in the solution 1 Daphnia remained in the normal state, 3 were greatly weakened, 2 were dead. In the above 3 weakened animals the eggs were laid: in one animal normally, but the eggs soon desintegrated into a shapeless mass, in two others the eggsubstance without having formed separate eggs passed into the moult of the abdomen and filled all its cavities. — Simultaneously in $\frac{n}{120}$ KCl-

solution the adult animals after 24 hours were in a quite normal state and the laying of eggs went on quite regularly.

6) Besides this were caried out 2 experiments with $CaCl_2$ solutions. The latter proved to be much less poisonous than isotonic KCl solutions. The animals submitted to these experiments were taken from the same aquarium as in experiments with KCl. The series consisted of 2 cultures: $\frac{n}{15}$ $CaCl_2$ and $\frac{n}{30}$ $CaCl_2$. $\frac{n}{15}$ $CaCl_2$ was strongly poisonous: after 4 hours all animals were dying, the eggs were not laid at all. In $\frac{n}{30}$ $CaCl_2$ the weakening of the adult was to be noticed on the second day of the experiment, the eggs were laid by 3 animals, but by two of them but partially and in the third of them not into the broodpouch but into the moult of the abdomen.

Comparing the results of the two last series with the preceding ones we cannot help seeing that the laying of the parthenogenetic eggs into the broodpouch is in close connection with the inner condition of the adult animal. The $\frac{n}{60}$ KCl- and $\frac{n}{30}$ $CaCl_2$ solutions stop or greatly disturb the egg laying function, whereas in NaCl solutions isotonic with the above solutions the eggs are laid quite regularly. Thus the outer osmotic pressure cannot be regarded in these cases as the hindering factor. This factor is to be sought in the condition of the female which is afflicted by the poisonous influence of the K-resp. Ca-ions.

The inner condition, according to the investigations of H. Fritzsche depends upon the osmotic pressure of the inner fluid in the organism of Daphnia. F. established that the height of this pressure shows remarkable oscillations depending on the age of the animal and on its different physiological state. It increases constantly before every casting of the moult and before every laying of the mature eggs into the broodpouch. These two functions, in biological respect quite different, in psysiological respect are connected with more intensive production of new organic matter. Both, after the casting of the moult and after the laying of the eggs, a distinct decrease of the inner pressure in the adult organism was found. In Daphnia these two psysiological phenomena appear almost at

the same time. — The height of the inner pressure in Daphnia organism in the period of time preceding either of these functions, or, more strictly, the preponderance of this pressure over the osmotic pressure of the surrounding medium is for both these functions a factor of great importance.

I noticed many times, that the casting of the moult in Daphnia meets with difficulty in higher concentrated solutions: either the moult is not cast in due time, or the old integument bursts in the dorsal neck region, the animal is too weak however to be able to get out of this integument. — Usually the laying of the eggs is disturbed too at the same time. It occurs sometimes, that after a certain retardation (several hours) the old integument is finally cast off, and therewith are cast the freshly laid eggs, which as a rule are not capable of any development. — The common cause of all these phenomena lies in the circumstance, that Daphnia being transferred from the fresh water to a solution is not capable of acquiring the necessary increase of the inner pressure to keep the same preponderance over the outer osmotic pressure as before the experiment. — If such an augmentation of the inner pressure takes place, one can regard the animal as acclimatised to the solution. H. Fritzsche describes experiments in which Daphnias were acclimatised to the solution, the osmotic pressure of their inner fluid was really higher than in Daphnias living in normal conditions, and their vital functions were not disturbed. — Often however the Daphnias are not capable of acclimatisation. The cause of this inability (according to H. Fritzsche) lies usually in the insufficient feeding of the animals; well fed animals have alvays higher inner pressure than those bred on insufficient nutriment.

In observed many times that the laying of the winter eggs was hindered by the influence of the sodium chlorid- and van't Hoff solutions too; this function was usually even more disturbed than the laying of the parthenogenetic eggs. — Once I noticed that in a Daphnia living in van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl- solution the ephippium was formed, 2—3 days afterwards it was cast off with the whole of the old integument, and the wintereggs, though mature, for some days could not leave the ovaries and remained there. I dit not investigate however the question of the wintereggs more particularly.

It may be thus accepted as proved that the laying of the parthenogenetic eggs depends directly on the inner condition of the adult animal, the criterion of which is the osmotic pressure of the inner body fluid. If the adult animal is enfeebled in whatsoever way, say by the poisonous influence of certain ions (K), or by the sudden increase of the osmotic pressure in the surrounding medium (experiments with sodium chlorid and Ringer- and van't Hoff's solutions), the inner osmotic pressure decreases and its preponderance over the outer pressure of the medium, if existing at all, remains insufficient, and the laying of the eggs is consequently disturbed.

The development of the parthenogenetic eggs in the salt solutions.

In the preceding chapter it was often mentioned, that the development of the eggs laid during the abode of the adult animals in the solutions passed irregularly or stopped just at the beginning. This phenomenon seemed to be quite independent of the condition of the adult animal as well as of the ability of the eggs to pass from the ovary into the broodpouch. Therefore it must be considered separately from the last function as something depending upon other factors.

The disturbing or even stopping of the development in the eggs of Daphnia is sometimes to be noticed in natural conditions. Among the eggs in the broodpouch of normal, in the pond water living, animals some appear unable to develop, whereas the rest of the brood pas through their development normally. The question of inability to develop in eggs of Cladocera in the normal conditions has been specially investigated by Woltereck, Papanicolau and Langhans. These three authors explain the fact by different causes. Woltereck finds in *Daphnia longispina* and *Simocephalus*, and Papanicolau in *Moina*, that the later is in the development cycle the brood and the generation to which the parthenogenetic eggs belong, the less they are able to develop and the more they are inclined to the degeneration. These authors thus accept the inner factors as the causes of the lack of vitality in eggs. — Langhans on the contrary accepts that the decrease of the ability to develop in the eggs of four, by him investigated, species of Daphnia is caused by the poisonous action of the products of change of matter, which

increase in quantity during the experiment. The cause should thus to be found in outer, chemical factors.

I noticed sometimes that the Daphnias taken from the pond and placed in less favourable conditions (great number of individuals in small quantity of water, insufficient food) grew weaker and parallely increased among the eggs laid into their broodpouches the number of incapable of development. Such eggs in a short time grew dark, degenerated and finally fell out of the broodpouch. The more the water evaporated the more the adults grew weak and died and the more the eggs contained in the broodpouches of the still living and apparently normal animals showed lack of development power.

On the other hand in the cultures of Daphnia kept in good conditions (water in sufficient quantity and relatively pure, abundant food in the form of minute algae) no lack of development power was to be seen. I found it in the aquariums of a larger size as well as in small pond water cultures belonging to the experimental series.

These observations prove that the disturbance in development in the Daphnia eggs can be caused by other factors besides the salt-solutions. On the basis of these observations it cannot be however stated, where the proper cause of the disturbing is to be found: in the inner condition of the adult animal, in the broodpouch of which lie the degenerating eggs, or in the direct influence of the surrounding medium, which may easily penetrate into the broodpouch.

I carried through a number of experiments which should contribute to the explaining of this question. On the one hand Daphnias originating from different natural cultures resp. aquariums were placed in solutions of identical chemical composition and concentration, on the other hand Daphnias of the same origin were placed in the series of solutions differing either in concentration or in chemical composition. — Besides the above mentioned question some others were connected with these experiments: in what degree the development of the eggs is depending on the concentration of the solution and what is the behaviour of the new generation eventually bred in the experimental conditions.

The experiments proved that the animals of different origin show

very different vitality, and their eggs very different capability of development in the same or similar solutions.

7) 30 young *Daphnia pulex* were taken from the aquarium in which they were bred as the first parthenogenetic generation of the animals derived directly from the wintereggs. The aquarium contained a strongly evaporated and condensed pond water. The young were small (1·3 mm length on an average), with colourless haemolymph, and their ovaries were filled up with maturing parthenogenetic eggs of a vivid bluegreen colour. Their movements were a little weakened. The animals of the preceding generation had been much bigger and with red hemolymph. This young Daphnias were distributed in 5 cultures: 1) pond-water (testculture), 2) tap water, 3) $\frac{n}{24}$, 4) $\frac{n}{20}$, and 5) $\frac{n}{15}$ NaCl solution. During the first day all animals showed a considerable weakening except the animals of the testculture. After 30 hours (from the beginning of the experiment) all animals, except those of the testculture, lay motionless. The eggs were laid besides the testculture only in $\frac{n}{24}$ NaCl and remained without development. It was a case of an extreme susceptibility to the solution, caused probably by the previuso weakening of the culture.

8) 40 young *Daphnia pulex* were taken from another aquarium. They were like those of the preceding series the first generation bred from the parthenogenetic eggs of the animals derived directly from the winter eggs. Like in the preceding series they were small sized, with the colourless hemolymph, whereas their mother generation consisted of animals much bigger (2·5 mm length on an average) with red hemolymph. The difference between the young Daphnias of this series and of the preceding one consisted in the fact, that they had lived in an aquarium with normal, not evaporated water. — They were distributed in 4 cultures: 1) pond water, 2) $\frac{n}{30}$, $\frac{n}{20}$, and 4) $\frac{n}{15}$ NaCl solution, 10 Daphnias in to each cultures Table IV).

Further observations showed that the adult animals remained alive for a longer time only in the testculture and in $\frac{n}{30}$ NaCl-solution, and the eggs developed only in the testculture.

Table IV.

	pondwater	$\frac{n}{30}$ NaCl	$\frac{n}{20}$ NaCl	$\frac{n}{15}$ NaCl
after 12 hours eggs laid in	6	6	6	4 (irregular)
condition of adult animals	all normal	all normal	all normal	slightly weakened
after 24 hours condition of adult animals	all normal	all normal	slightly weakened	weakened
after 36 hours eggs laid in	10	10	10	10
condition of adult animals	all normal	all normal	all weakened	all weakened
development of the eggs	normal	no development or only up to primitive stages	no development	no development

This remarkable difference in vitality and reproduction power between the two desribed series [7) and 8)] in favour of the latter can be ascribed to the better conditions in which the animals of the 8) series had lived before the experiment. Although however the resistance power in the adults to the solutions was great enough, the eggs were not able to develop. It was especially evident in the culture in $\frac{n}{30}$ NaCl, in which the adults remained in a normal state for a longer time. — Other experiments proved however that in weaker concentrated solutions the development goes on quite normally, and that the rate of development is the same as in pond water. This was found in experiments carried out on Daphnias as well as on other related forms e. g. *Scapholeberis mucronata.* (Pl. I. Fig. 8).

9) 20 *Daphnia pulex* were taken from an aquarium abounding in minute, unicellular algae. Daphnias appeared during the winter (February) probably developed from the wintereggs. The animals were large sized (about 2·5 mm length), with a red hemolymph, well fed, and were in the period of an intensive production of parthenogenetic eggs (20—30 eggs in one brood). The above 20

animals were of about the same size and with the eggs in the same stage of development (mature eggs in the ovaries and older embryos in the broodpouch). They were distributed into 5 cultures: 1) aquarium water, 2-3) $\frac{n}{20}$ NaCl, and isotonic van't Hoff's solution, 4-5) $\frac{n}{10}$ NaCl, and isotonic van't Hoff's solution.

Table V.

	aquarium-water	$\frac{n}{20}$ NaCl	van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl	$\frac{n}{10}$ NaCl	van't Hoff's solution isotonic with $\frac{n}{10}$ NaCl
after 24 hours eggs laid in	2	2	2	—	—
condition of adult animals	all normal	all normal	all normal	slightly weakened	slightly weakened
after 72 hours eggs laid in	4	4	4	—	—
condition of adult animals	all normal	all normal	all normal	all dead	all dead (lived a little longer than in $\frac{n}{10}$ NaCl)
after 84 hours condition of embryos	in the endstage with unpaired eye	in the endstage with unpaired eye	in the endstage with unpaired eye	—	—
after 108 hours condition of embryos	swimming free in the water	swimming free in the solution	swimming free in the solution	—	—

The above young Daphnias of the second generation, bred in the test culture, in $\frac{n}{20}$ NaCl and in the isotonic van't Hoff's solution, were transferred into larger vessels with the same solutions resp. with water from aquarium, in which they had lived hitherto, and supplied abundantly with small algae.

Some young Daphnias died during the further experiment, the rest however lived and grew in all three cultures normally. In the cultures with $\frac{n}{20}$ NaCl and with coresponding van't Hoff's solution on the 15-th day of the life of the young Daphnias (reckoning from the day when the eggs, from which they developed, had passed into the broodpouch) first parthenogenetic eggs of the new generation appeared, but only in some individuals. — During the three following days the eggs passed in all remaining females of these two cultures into the broodpouch. — In the testculture however the eggs passed into the broodpouch in all Daphnias during the 18-th day of their life only. In this case the maturity was thus attained earlier in the animals bred in the solutions than in those bred in the aquarium water.

On the basis of observations concerning these 3 cultures of the second generation the following facts may be stated:

1) the number of parthenogenetic eggs in single broods in all three cultures was the same (6—8, exceptionally 11).

2) the eggs were laid in all three cultures regularly (except in 2 individuals in van't Hoff's solution).

3) all these eggs (of the third generation) developed normally (except the few which had been laid irregularly) and their total embryonal development lasted in all three cultures about 80 hours.

4) in all three cultures the first brood was followed by the regular laying of the second brood after the same interval of time.

5) the eggs of the third generation when left in their experimental solutions, developed normally like the eggs of the second one, and the embryos reached their maturity within the same time.

10) From the same aquarium as for the 9. series Daphnias were taken for a series of similar experiments consisting of 3 cultures: one testculture (aquarium water) and two cultures with van't Hoff solutions isotonic with $\frac{n}{20}$ and $\frac{n}{10}$ NaCl solutions. The animals did not differ in any way from those of the 9. series.

Daphnias in the vant' Hoff's solution isotonic with $\frac{n}{10}$ NaCl died all during the third day of the experiment, in two other cultures many Daphnias died too, those however which remained alive, developed parallely both in the pond water and in the van't Hoff's

solution isotonic with $\frac{n}{20}$ NaCl solution. The first parthenogenetic eggs appeared in the Daphnias of both cultures in their 15-th resp. 16-th day of life. In this series there was not to be noticed any perceptible difference in the time necessary for the attaining of maturity between the animals bred in the water and the preceding series.

Having ad my disposal animals bred normally in the $\frac{n}{20}$ NaCl solution and in the van't Hoff's solution of the same concentration, I tried to find out whether the eggs produced in them, are capable of development in higher concentration than the eggs of the preceding generation. In these experiments I was limited by the small number of animals of the second generation bred in the solutions, which remained alive. — As the next higher concentrated I chose $\frac{n}{15}$ and $\frac{n}{12}$ NaCl solution and isotonic van't Hoff's solutions.

11) 2 young Daphnias from the testculture (aquarium water) and 1 Daphnia from $\frac{n}{20}$ NaCl solution, all three from the second generation of the 9. series, and all three with maturing eggs in their ovaries, were placed (separately, in two vessels) in $\frac{n}{15}$ NaCl solution. The eggs were laid soon into the broodpouches in all three animals (5—6 eggs in every brood). In each brood some eggs remained without any development, some developed abnormally, (without the bursting of the eggmembrane), some developed normally. The normally developing embryos in all three Daphnias passed through their development in about 80—90 hours.

Thus the development in $\frac{n}{15}$ NaCl-solution lasted for an equally long space of time in embryos of the Daphnias bred in $\frac{n}{20}$ NaCl solution and in those of the Daphnias bred in the water, besides, it lasted the same lenght of time as the development in the $\frac{n}{20}$ NaCl solution and even as the development in the aquarium water.

After the embryos of the first brood had left the broodpouch, the new eggs were laid by all three animals, and their development

lasted for the same lenght of time as the development of the first brood. — Thus in this experiment I could not find any difference in the lenght of the development period in $\frac{n}{15}$-solution between the eggs derived from the testculture with aquarium water and those derived from $\frac{n}{20}$ NaCl-solution.

I must add however that a normal development in $\frac{n}{15}$ NaCl solutions in eggs, the layers of which had lived in the water before the laying of the eggs, occured in my experiments very seldom.

12) 2 young Daphnias from the testculture (water from aquarium) and two from the $\frac{n}{20}$ NaCl solution, all 4 from the same second generation of the 9. series, all four with maturing eggs in their broodpouches, were placed (each party separately) in $\frac{n}{12}$ NaCl solution. In both cultures eggs were laid, but remained mostly without development and degenerated. Only in one Daphnia derived from $\frac{n}{20}$ NaCl one embryo reached the stage of paired eyes but remained closed in the eggmenbrane and soon died.

13) 2 young Daphnias from the testculture and 2 from the van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl, belonging to the second generation bred in the 9. series and with maturing eggs in their broodpouches (of the same origin as Daphnias used in series 11. and 12.) were placed (each party separately) in van't Hoff's solution isotonic with $\frac{n}{12}$ NaCl.

The effect of the experiment was similar to that of the 12. series: the eggs were laid into the broodpouch in all four Daphnias; for the most part they remained without development, except three laid by animals taken from the testculture, which attained the same stage of paired eyes as in the foregoing series and perished soon, closed in the eggmembranes.

Thus in the series 12. and 13., similarly as in the series 11., no distinct difference was to be noticed in the development power, in a higher concentration, between the eggs of the animals bred in

$\frac{n}{20}$ NaCl solution or isotonic van't Hoff's solution, and the eggs of the animals bred in aquarium water. — As to the adult animals they lived in the solutions for several days, afterwards they weakened. After the normal interval of time new eggs were laid in the $\frac{n}{12}$ NaCl- and isotonic van't Hoff's solution, but they showed complete lack of development.

Some experiments aimed at discovering whether on the contrary the eggs, laid in a solution, transferred into water, show a change in their development power.

14) 8 *Daphnia pulex* were transferred, after the laying of the eggs, from $\frac{n}{20}$ NaCl-solution into pond water. — In 4 individuals all eggs remained without development, in 2 others all eggs developed normally, and in 2 individuals some eggs of the brood developed normally and some remained without development.

5 other Daphnias were transferred from the same $\frac{n}{20}$ NaCl-solution into pond water, just before the laying of the eggs into the broodpouch, the eggs were laid in them after the transfering into pond water. In both cultures in all eggs in which the development did not stop at beginning, the development proceeded quite parallely. It was parallel too to the development of the eggs of the same age remaining in the testculture with pond water.

The results of the above described cultures of the series 7—14 can be summarised as follows:

1) The sodium chlorid-, Ringer- and van't Hoff's solutions checked the development of the parthenogenetic Daphnia eggs laid into the broodpouch during the abode of the adult animals in these solutions. — This checking influence was to be noticed usually in $\frac{n}{30}$ NaCl (or isotonic van't Hoff's solution), increased parallely to the concentration, and in the concentrations above $\frac{n}{12}$ NaCl was strong enough to stop the development completely in the outset.

2) In the cultures bred in the solutions appeared remarkable differences in the development power among the eggs of Daphnia. These differences may occur among different populations of Daphnia,

as well as among different individuals, of the same population, as among different broods of the same individual, as finally among the eggs of the same brood.

3) The development power in Daphnia eggs in the solution seemed to depend in some measure also upon the previous condition of the adult animal in the period when these eggs were maturing in the ovaries, and when their better or worse supplying with reserve materials depended on the condition of the adult animal.

4) The new (second) generation of Daphnia, bred from the eggs, which had been laid in $\frac{n}{20}$ NaCl- or isotonic van't Hoff's solution, when left in the same solutions attained its maturity i. e. began to produce the parthenogenetic eggs either in the same time as Daphnias of the same age bred in pondwater (series 10.) or 1—3 days earlier (series 9.). The next following (third) generation, if bred further in the same solution, attained its maturity in the same time as the second generation.

5) The parthenogenetic eggs of the second generation, bred in $\frac{n}{20}$ NaCl- resp. isotonic van't Hoff's solution, were laid in the broodpouch and developed regularly almost all, as contrasted with the eggs of the first generation, from which only few developed normally in the same conditions.

6) The rate of the embryonal development is as long in the embryo bred in pond water as in NaCl-solutions up to the concentration $\frac{n}{15}$, as in the isotonic van't Hoff's solutions.

Dimensions and development ability in the eggs of different broods in solution.

In some of the cultures already described and in some others too I observed the development of two or three subsequent broods in the same individuals. It was possible only in less concentrated solutions, which allowed the animals to live at least 4 days or longer. (The normal development of the eggs up to the endstage of embryonal life lasted in my cultures about 75—85 hours, and the time interval between the moments of passing into the broodpouch of two sub-

sequent broods a few hours longer). In such cultures following facts were observed:

1) If the adult Daphnia despite the action of the solution remained in the normal state, the first brood was followed by the second after as long an interval of time as in the pondwater culture, if however the adult Daphnia was weakened, the laying of the second brood was retarded (simultaneously the casting of the integument was retarded, as previously mentioned). Sometimes the eggs of the second brood met in the broodpouch with the eggs of the first brood, the development of which had stopped. The eggs which had passed into the broodpouch during the abode of the adult in a stronger concentrated solution $\left(\frac{n}{12}\ \mathrm{NaCl}\right)$, often stopped in their development at a primitive stage, if however the adult Daphnia in spite of the solution remained in a normal condition, the intervals between the following broods were as long as in Daphnias living in the pondwater. This fact seems to prove once more, that the experimental solution does not exercise an immediate influence on the formation and maturing of the eggs in the ovaries.

2) If the experimental solution was strong enough to check the development of the eggs of the first brood at an early stage, the development of the eggs of the following broods usually did not overpass that stage either. The development power in the eggs of following broods in the solutions on the contrary seems to decrease the lower the later the brood. This fact, concerning the behaviour of Daphnias in the solutions, harmonises with the facts found by Woltereck and Papanicolau in *Daphnia*, *Moina* and *Simocephalus*, as previously mentioned, that in normal conditions too the vitality of the eggs decreases in following broods, the later the brood the oftener the eggs appear with an abnormally small quantity of yolk, of worse quality, and unable to develop.

3) The eggs of the following broods, laid in the solutions, do not seem to differ as to the average size. The eggs of the following broods must be measured obviously in the same stage of their development, whereas the dimensions increase in each developing egg both in normal and in artificially changed conditions up to a given moment. The abnormally small eggs, appearing sometimes in the solutions cannot naturally be taken into consideration.

That the average size of the eggs does not vary from brood

to brood in the same individual living in the solution following figures may serve as an example:

In one *Daphnia pulex*, living in the $\frac{n}{30}$ NaCl culture, all eggs of three following broods in the stage of blastoderm were measured. (Pl. I. fig. 9)

eggs of the I brood — longer axis 0·235—0·283 mm, shorter axis 0·168—0·186 mm,
II brood — longer axis 0·247—0·271 mm, shorter axis 0·162—0·174 mm,
III brood — longer axis 0·259 mm, shorter axis 0·162 mm.

The eggs of the I brood ceased to develop in the stage of the paired eyes, the eggs of the II brood in the earlier stage of the formation of extremities, the eggs of the III brood in the stage of blastoderm.

In one culture of *Ceriodaphnia* however, in the van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl solution, the eggs of three following broods differed distinctly in their dimensions (fig. 2). All eggs produced by one Ceriodaphnia during abode in the solution were measured. The dimensions are naturally smaller than in the Daphnia eggs, as *Ceriodaphnia laticaudata* herself is much smaller than *Daphnia pulex*.

I brood — longer axis 0·174 mm, shorter axis 0·114 mm,
II brood — longer axis 0·229 mm, shorter axis 0·139 mm,

the eggs of both broods were measured in the stage of the paired eyes (in which the embryos remained however closed in the eggmembrane) and which was the final stage of their development. In the eggs of II brood in the measured stage between the eggmembrane and the body of the embryo appeared a liquid substance orange coloured which in its colour resembled the hemolymph of the same species.

Length of the embryo inside the egg 0·20 mm.

Thus the difference in dimensions between these two broods was considerable. This example does not confirm the rule that the eggs of subsequent broods must be equal in size.

The eggs of the II brood were transferred into distilled water. — This was done without injuring the adult female as the eggs had been previously expelled outof the broodpouch with the last moult

as it sometimes happens in case of abnormal development. The adult animal remained alive further in the solution. The eggs placed in the distilled water showed during the first hour no change in dimensions, during the second hour however their volume began

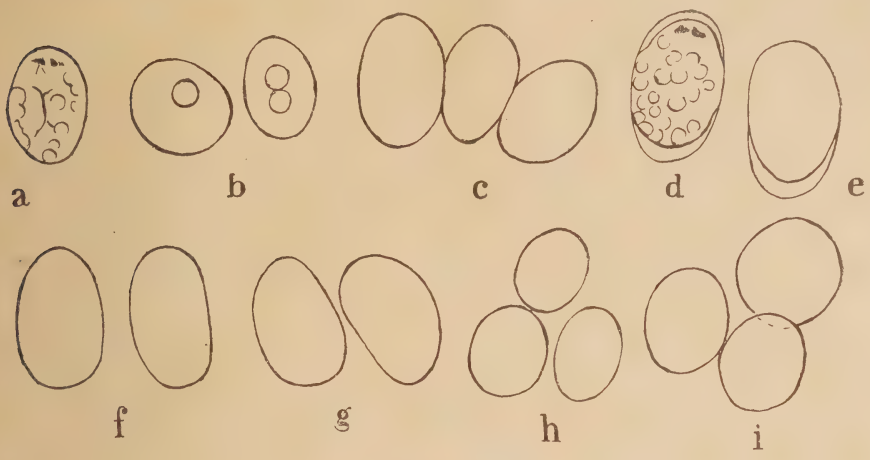

Fig. 2.

Parthenogenetic eggs in 3 subsequent broods in *Ceriodaphnia laticaudata* in van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl; a) eggs of the first brood in endstage („closed" development); b—g) eggs of the second brood, b) immediately after passing into the broodpouch, c) the same 24 hours later, d) the same in the stage in which their development ceased, e) the same 40 min. —, f) 135 min. —, g) 24 hours after transfering into the distilled water; h—i) eggs of the third brood, h) after passing into the broodpouch, i) after 96 hours abode in the solution.

to decrease and the orange coloured fluid gradually disappeared. After about $2^1/_4$ hours (from the moment of transfering into distilled water) the fluid disappeared completely and the eggmembrane adhered tightly to the embryo. The longer axis of the egg in that moment was 0·21 mm. No further development of the embryo was noticed and during the following day the size of the egg still slightly decreased (longer axis 0·20 mm).

III brood (of the same Ceriodaphnia): longer axis 0·17 – 0·18 mm, shorter axis 0·12—0·15 mm.

These eggs remained without any development and without the secreting of a coloured fluid.

A similar difference in the dimensions of the eggs in the same

stage of development I observed in a culture with *Pleuroxus aduncus* in $\frac{n}{15}$ NaCl-solution: (fig. 3)

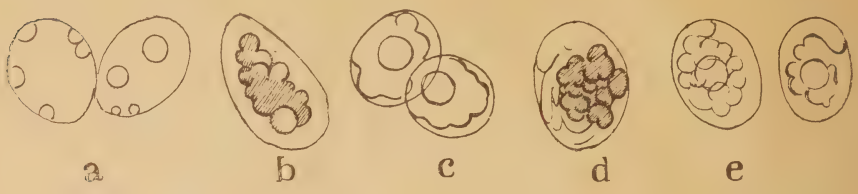

Fig. 3.

Parthenogenetic eggs of *Pleuroxus aduncus* — restraining of development in various stages; a—b) in $\frac{n}{15}$, c—e) in $\frac{n}{20}$ NaCl solution.

in eggs normally developing longer axis 0·15—0·17 mm,
in eggs with secreted fluid longer axis 0·24 mm.

The latter remained without development. The above broods did not belong however to the same adult individual.

Differences in development ability in the presence of the solution among the eggs of the same brood.

In connection with experiments hitherto described I want to emphasise the frequent recurrence of remarkable variety in development ability and resistibllity of the eggs in the cultures with solutions. The series described up to now prove that the animals derived from different ponds or aquariums behave in the solutions variously in ability of reproduction and in development ability of their eggs. This fact may be explained both by different outer and inner conditions in different natural cultures influencing the value of the inner osmotic pressure in Daphnias as well as influencing the better or worse supplying of their eggs with reserve materials, as was proved by H. Fritsche.

By the variety of outer conditions however may be explained only the differences in the vitality in eggs of different natural cultures. The differences in the vitality of the eggs in the same natural culture can be explained rather by the belonging of the eggs to different subsequent broods and generations in the development cycle. These differences are however in the cultures, living

in prosperous normal conditions and abundantly fed, usually very slight, especially if all individuals contained in one culture are in the same age.

On the contrary, in the experimental cultures in the saltsolutions appear remarkable differences in development ability not only in eggs of different animals but also in eggs of the same brood in the same animal. Simultaneously eggs of animals derived from the same natural culture as those of the saltsolution, when bred in pondwater, show no such differences in development. The differences in eggs in the solutions are especially well to be observed in big animals with a great number of parthenogenetic eggs laid as one brood into the broodpouch.

Example. In 1 Daphnia pulex from the 12. series (van't Hoff's solution isotonic with $\frac{n}{15}$ NaCl) 21 eggs were laid into the broodpouch. Three days later: 12 eggs remained without development, 3 passed through their development closed in the eggmenbranes, and 6 passed through the normal development and were about to leave the broodpouch. In other individuals I noticed some embryos stopped in earlier stages of development, while other embryos from the same brood developed normally. (Pl. I. fig. 10).

The researches of St. Przyłęcki, based on his measurements of the dimensions and of the inner osmotic pressure in Daphnia- and Simocephalus eggs, proved that among the eggs of the same brood in normal conditions exist some oscillations, both in length of axis and in height of the inner pressure. Despite these oscillations all eggs living in normal conditions in the same culture develop parallely as a rule. The abnormal conditions existing in the artificial solutions seem to emphasise these slight almost imperceptible differences and to produce a visible effect.

The total embryonal development within the eggmembrane.

In the description of my experimental cultures sometimes I mentioned, that the embryos of Daphnia passed through their total development closed in the eggmembrane. Such a development was described in my previous work from 1914. (Chapter VIII.). This development could be easily evoked, when an adult Daphnia with eggs in the broodpouch in an early development stage was transferred

from normal culture (pondwater) to the NaCl solution. This phenomenon could be evoked in eggs too, when they were transferred alone (brought artificially outof the broodpouch) from pondwater into solution. Such development I had observed too in the parthenogenetic eggs transferred from pondwater to cane sugar solutions isotonic with NaCl-solutions.

Between the normal development of the Daphnia embryos and this „closed development" exist some differences. The normal development of the embryos of *Daphnia magna* and *Simocephalus vetulus* was described by St. Przyłęcki in his work about the osmotic pressure in parthenogenetic eggs. — During the embryonal life the membranes surrounding the embryo burst twice: first the eggmembrane, then the larval membrane. The development of the embryo, i. e. the formation and the differentiation of the organs, goes on continuously, the growth however is not continuous. In the first phase, inside the eggmembrane, the embryo grows as long a time as it is allowed by the expansibility of the membrane, then the growth stops for a certain time, then in consequence of the increasing inner pressure, the eggmembrane bursts, the embryo gains rapidly in volume, then the rate of the growth decreases again and growth goes on further in a more uniform manner. During the latter phase the larval membrane bursts.

During the „closed development" the growth of the embryo is limited by the existence of the not bursting eggmembrane. This growth coresponds in its extension to the first phase of the normal growth, afterwards it stops altogether. The embryo remains closed in the eggmembrane. In spite of this closure and of the impeding of growth the embryonal development goes on almost parallely to the normal development of other embryos, in which the eggmembrane had burst in due time. It can be distinctly confiirmed in such cases, when the eggs of the same brood some develop normally and others go through a „closed development". The parallelism of both these forms of development does not concern however all processes taking place in the embryos. Simultaneously, both in normal and in „closed" development, are formed antennae, extremities, paired eyes (joining usually in the same time into the unpaired eye), the heart is formed and begins to beat in about the same time in the normal and in the closed embryo. — The amount of reserve materials however (yolk and fat) in the later stages of

development seems to be greater in the closed than in the normal embryos, the change of matter in the closed embryos is probably less intensive and the consumption of the reserve-materials goes on slowler. — The single ball of fat which during the normal development decomposes in the embryo usually into several smaller balls, remains undivided during the total development. — Sometimes a certain retardation in comparison with normal embryos may be noticed in the closed embryos in the formation of the unpaired eye, in which the pigment seems to prevail over the light breaking elements. The heart at the beginning of its pulsation in the closed embryo is sometimes red coloured whereas in the normal embryos of the same culture it is of a pale colour. It seems as though its colour were caused by the condensed colouring substance of the hemolymph. (Pl. I. fig. 11.).

If the embryo overpassed in the „closed" development the stage in which normally the eggmenbrane bursts, it remained usually in its closure up to the end. The spontaneous bursting of the membrane in a later stage occurred but seldom. In embryos, which had attained the stage coresponding to the stage in which the normal embryos leave the broodpouch, the eggmembrane never is seen to burst spontaneously, the development ceases, all vital functions are weakened, the pulsation of the heart, which in the „closed" development can amount to 150 and more in a minute, gradually ceases too, the movements of the antennae and of the abdomen, which are often visible in the later stages within the eggmembrane stop too, and the embryo dies in 4—5 days from the time when the egg had passed into the broodpouch.

The „closed" development can be artificially interrupted in two ways. One method consists in the decreasing of the osmotic pressure of the surrounding medium. I brought the embryos being in the endstage of the „closed" development outof the broodpouch and transferred them from the saltsolution to a fluid of a much lower osmotic pressure e. g. to pond-, tap- or distilled water. After a certain time (usually after 10—20 hours) the eggmembranes burst, the liberated embryos however for the most part were not capable of further development and soon died.

Example of the „closed" development and of its artificial interruption by decrease of the osmotic pressure:

15) 9 *Daphnia pulex*, each containing in its broodpouch 6—10

eggs in the blastoderm stage, were placed in the van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl-solution. After 40 hours the embryos in all 9 Daphnias attained the stage of paired eyes, but in 5 Daphnias all embryos developed closed in the eggmembrane, in 4 other Daphnias some embryos developed normally, others were closed in the eggmembrane; after 64 hours all embryos in the cultures in the same but coresponding later stage of development, after 86 hours the closed embryos as well as the normally developed ones were in the endstage of the embryonal development. — The total number of closed embryos in that culture amounted to 60. Only in 2 of them eggmembrane burst spontaneously.

16) From another culture of *Daphnia pulex*, bred like the preceding in the van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl-solution, but containing the animals with greater broods (20—30 eggs in a broodpouch), one Daphnia was taken out after 64 hours abode of the Daphnias in the solution. Its eggs, or properly said, its embryos being in the endstage of the closed development, 24 in all, were brought outof the broodpouch and distributed: 1) 8 to the distilled water, 2) 8 to the pond water and 3) 8 to the same van't Hoff's solution, in which the mother animal lived. After 20 hours: 1) in distilled water: in all 8 embryos the eggmembrane burst, embryos dead and desintegrated (the membranes burst in the time from 10—20 hours); 2) in pond water: in 5 embryos the membranes burst, the fresh appearance of the embryos allows us to suppose, that the membranes had burst a short time previously; the embryos were not alive; in 3 other embryos the eggmembranes did not burst; 3) in van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl the eggmembrane did not burst at all. The result of this experiment may prove, that the bursting of the eggmembrane depends strictly upon the height of the osmotic pressure in the surrounding medium.

The other method of the interrupting of the „closed" development consisted in a cautious perforation of the eggmembrane by means of a needle. If certain precautions were taken the embryo could be extracted from the artificially split eggmembrane still closed in the undamaged larval mambrane. The embryo lived on and its further development depended upon two moments: 1) upon the nature of the

medium in which it was placed after its release from the eggmembrane, 2) upon the moment in which the release itself had taken place. — Description of the experiments below will explain this question.

17) A culture of *Daphnia pulex* in van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl was bred simultaneously with the culture 15. and did not differ from the latter in regard to the development of the embryo. From this culture 64 hours from its initiation one Daphnia was taken out and from its broodpouch were taken 10 embryos closed in the eggmembranes. All 10 were in the stage of the unpaired eye, showed fully visible movements inside the membrane and the heart pulsation in them was 40—50 a minute. These embryos were distributed into 3 fluids: 1) pond water, 2) van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl, and 3) van't Hoff's solution isotonic with $\frac{n}{10}$ NaCl. — Immediately after their transferment into these fluids the eggmembranes were perforated in all embryos with a needle and the embryos remained under further observation. (Pl. I. fig. 12).

The larval membranes, in which originally all embryos were closed, burst in the pond water culture in the course of the first 4 hours but remained unburst in all embryos living in the solutions.

Table VI.

	pond water	van't Hoff's sol. isot. $\frac{n}{20}$ NaCl	van't Hoff's sol. isot. $\frac{n}{10}$ NaCl
after 4 hours	larval membrane burst in all embryos	larval membrane remained in all embryos	larval membrane remained in all embryos
after 24 hours	embryos in normal condition endstage with mucro	embryos in normal condition endstage with mucro	embryos weakened slight retardation in development
after 48 hours	young normal	young normal	young (embryos) much weakened, growth ceased
after 72 hours	young normal	young weakened	young dead

Young Daphnias in pond water and in van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl grew and thrived normally for a longer space of time. (Pl. I. fig. 13).

18) From the same culture as in series 17. (van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl) 24 hours later was taken another Daphnia. She contained in her broodpouch 8 embryos closed in the eggmembrane like the embryos in series 17. These embryos derived from eggs laid in about the same time as the embryos from foregoing series. Thus at the beginning of the experiment 18. they were 24 hours older than embryos from series 17. at the beginning of their experiment. They were brought out of the broodpouch 88 hours after the moment of passing from ovaries into the broodpouch. Normal developing embryos in this age are already free swimming. — They did not show any more movements inside the eggmembrane. They were distributed into the same three fluids as the embryos of the preceding culture, and immediately after the transferment were liberated from the eggmembranes by a cautious perforation of the latter with a needle.

The development in that series turned out much worse than in series 17.; properly said there was no development at all. In all three cultures the embryos were much weakened, growth was unnoticeble, in the pond water and in the van't Hoff's solution isotonic with $\frac{n}{10}$ NaCl the pulsation ceased, in van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl it was indistinct. All embryos died in 24—30 hours after tbe release from the eggmembranes.

19) From the same culture as in series 17. and 18. was taken the third Daphnia 24 hours later than Daphnia of the series 18. From the embryos extracted outof the broodpouch, closed in the eggmembranes, as those of the foregoing series, only two were alive. Heart pulsation in them was about 75 a minute. The same stage of development as in series 17. and 18. As the eggs from which they developed had passed into the broodpouch in the same time as in foregoing series, their age was at the beginning of this experiment 112 hours. They were liberated from the eggmembranes by perforation and left in the same fluid (van't Hoff's solution isotonic

with $\frac{n}{20}$ NaCl). Neither growth nor further development was noticed in them. For several hours they showed pulsation and distinct though feeble movements. They ceased to live during the first day.

From the above experiments is evident that the later embryo is liberated from the eggmembrane, the weaker is his vitality and development power even in normal conditions.

In order to obtain a clear idea of growth during the „closed" development a series of measurements were made on eggs and embryos of the foregoing 3 series.

As regards the volume of the parthenogenetic Daphnia eggs in normal conditions, the measurements of Przyłęcki proved that it increases during the first 24 hours of normal development. This increase stops when the limit of expansibility of the eggmembranes is attained. The volume of the egg during the normal development has thus two limits: minimum in the beginning stage, just after the assuming of the definite shape, and maximum, which is defined by the expansibility of the eggmembrane. This maximum is attained in eggs of *Daphnia magna* (which was investigated by Przyłęcki) after 24 hours. In *Daphnia pulex* (which was the object of my researches) the growth of the eggs is quite analogical, the whole development lasts a little shorter.

My measurements proved, that the volume of Daphnia embryos in the endstage of the „closed" development attained usually the maximum defined by the expansibility of the eggmembrane.

Dimensions of the eggs, from which developed embryos which were used in series 17.—19., in their initial stage after they had assumed their definite shape and after their membranes had been formed, were: longer axis 0·229 mm, shorter axis 0·168—0·174 mm.

Dimensions of the same eggs 70 hours later, in the endstage of their closed development, just before the porforation of the membrane, were: longer axis 0·241—0·277 mm, shorter axis 0·18—0·21 mm. (Pl. I. fig. 12). The last dimensions are about the same as the dimensions of normally developing eggs just before bursting of the eggmembrane i. e. in the stage of the formation of extremities. Sometimes I met however the closed embryos, the volume of which did not differ from the volume of the normal eggs in their begin-

ning phase of development; these eggs seem not to attain the limit of the eggmembrane expansibility.

The further measuments concern embryos after the perforation of the eggmembrane. (Pl. I. fig. 14—16). The embryo before bursting of the egmembrane represents an elipsoid and its volum can be exactly given in cubic units if the longer and shorter axis are known. ($v = ab^2$). After the bursting of the eggmembrane its volume can be still defined by the length of its longitudinal and transversal axis, and its volume increases parallely with these axis though it is difficult to give it exactly. After the bursting of the larval membrane the embryo is no more a simple geometrical form; the measurements in this stage concerned only the body length from the forehead till the basis of the mucro.

The embryos of Daphnia near the endstage of the „closed" development (70 hours of age) after the perforation of the eggmembrane increased suddenly in volume. Outof the eggmembrane split into two equal, rapidly involving parts the nearly ovalshaped embryo emerges closed in the larval membrane. After the rapid increase of volume dimensions of embryos: length 0·277—0·289 mm, breadth 0·192—0·200 mm.

The embryo continues to grow but slowly and uniformly, the intensity of the further growth depending upon the concentration of the fluid in which the embryo is placed (s. Table VII).

Table VII.

	pond water	van't Hoff's sol. isot. $\frac{n}{20}$ NaCl	van't Hoff's sol. isot. $\frac{n}{10}$ NaCl
after 4 hours	length 0·361 · 0·385	length 0·355—0·385 breadth 0·21	length 0·283—0·319 breadth 0·20
after 24 hours	length 0·433	length 0·437—0·451	length 0·397
after 48 hours	length 0·570—0·638	length 0·494—0·530	length 0·361—0·421

Two dimensions (length and breadth) are given only of these animals (embryos) which remained still closed in the larval membranes.

Simultaneously the embryos from the same original culture

(van't Hoff's solution isoton. $\frac{n}{20}$ NaCl) exactly of the same age, which had passed however through a normal development, were measured.

In the moment of the last measurement of the above abnormal embryos (48 hours after the beginning of the experiment) length of the normal embryos: 0·554—0·650 mm.

The embryos from the 18. series, which had been liberated from the eggmembrane 24 hours later, were measured too.

Table VIII.

	pond water	van't Hoff's sol. isot. $\frac{n}{20}$ NaCl	van't Hoff's sol. isot. $\frac{n}{10}$ NaCl
after 4 hours	length 0·409	length 0·343—0·361	length 0·337

No further growth took place.

In the embryos from series 19., which had been liberated from the eggmembrane 24 hours later than in series 18. and which had lived for one day only, 4 hours after the perforation of the eggmembrane: length 0.295—0·319 mm.

The growth of the *Daphnia pulex*-embryos during the „closed" development and after its artificial interrupting is graphically presented in fig. 4). In the same figure for comparison the growth of the embryo of the same species during the normal development is delineated. As it is evident from the figure, the curves of the growth for both kinds of development meet at the end of the fifth day of the embryo life, if the embryos after the interrupting of the „closed" development were transferred into the pond water.

The „closed" development was as a rule evoked in Daphnia eggs by putting them in an early stage, yet after the eggmembrane had been already formed, into saltsolution. These eggs had been laid during the abode of the adult animals in the pond-, resp. aquarium water. The „closed" development occurred however in the eggs too, which had been laid during the abode of the adult animals in the salt solution. The percentage of the abnormally developing eggs (closed in the eggmembrane) is in the latter case however much smaller than in the eggs laid before transfering into

the solution. We can however observe the closed development in eggs laid into the broodpouch in the presence of the saltsolution only in lower concentrations (up to $\frac{n}{12}$ NaCl) on account of their-

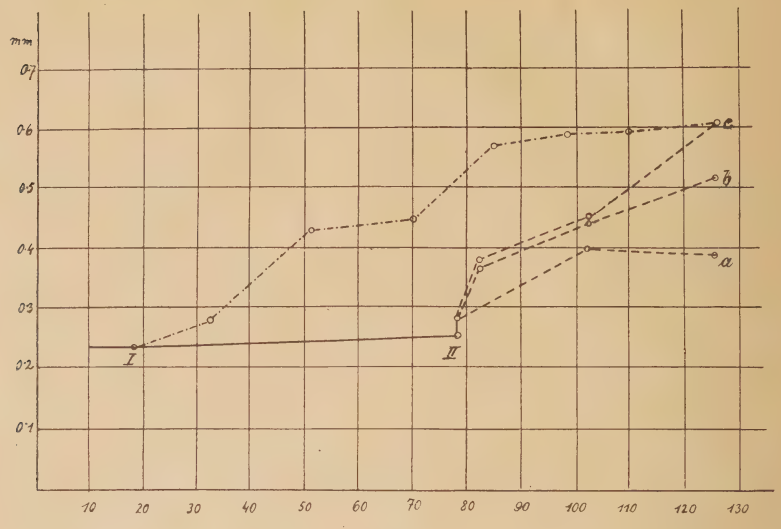

Fig. 4.

Graphic delineation of the growth rate in the embryos of Daphnia pulex after the artificial interruption of the „closed" development in van't Hoff's solutions of various concentration in comparison with the normal growth in pond water. The full line denotes growth during the „closed" development in van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl. I-moment of bursting of the eggmembrane during the normal development, II-moment of artificial interruption of the „closed" development. Lines of dashes denote further growth after the interruption, *a*: in embryos transferred immediately before the interruption of the „closed" development into van't Hoff's solution isotonic with $\frac{n}{10}$ NaCl, *b*: in embryos left in that moment in van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl, *c*: in embryos transferred in that moment into pond water. — The line combined of dashes and dots denotes growth of embryos during the normal development.

great sensitiveness in the first membraneless stage to the action of ions, which makes impossible their development in concentrations above $\frac{n}{12}$ NaCl; whereas the eggs laid into the broodpouch in the presence of pond water and transferred into the solution

some hours later when protected already by a solid eggmembrane, can sustain during the closed development much higher concentration (up to $\frac{n}{6}$ NaCl).

In my experiments eggs which passed through the closed development in $\frac{n}{12}$ NaCl (higher concentration than in series 17.—19.) were not capable after liberation from the membrane of a quite normal development.

20) Example. — (Pl. I. fig. 17). Amongst the eggs which were laid into the broodpouch after the transferring of the adult animals from $\frac{n}{20}$ NaCl into $\frac{n}{12}$ NaCl only one egg developed up to the stage of paired eyes. Dimensions of this egg were in that stage (which seemed to be its endstage): length 0·277 mm. breadth 0·180 mm.

The embryo was taken outof the broodpouch and liberated immediately afterwards outof the eggmembrane.

6 hours later — length of the embryo 0·289 mm.

24 hours later — length „ 0·30 mm.

The embryo attained the stage of the unpaired eye, and neither growth nor development was any more noticed. If compared with the measurements from the preceding experiments (in $\frac{n}{20}$ NaCl) growth seems to be very limited.

I have no record of a closed development in eggs developing in KCl-solutions. The cause seems to lie in the circumstance that potassium chlorid in consequence of its much more poisonous effect could be used only in lower concetrated solutions (up to $\frac{n}{60}$ KCl). Adult Daphnias with the developing eggs in their broodpouches were transferred into $\frac{n}{60}$ KCl solution from the pond water. This solution was poisonous enough to kill the animals in 5—7 hours, the eggs however develop normally, the eggmembrane as well as the larval membrane burst in due time, and the embryos attained the independent stage during about 80 hours like the embryos developing in the pond water. The poisonous effect of KCl was to be noticed at first in young individuals swimming freely.

The experiments with KCl solutions evidently prove that the

phenomenon of the „closed“ development depends only on the height of the osmotic pressure of the medium, in which the eggs are placed, and not on the chemical nature of it. Not excepting those cases when the medium exercises a poisonous effect on the adult animal. That the form of development depends on the osmotic pressure i. e. on the concentration of the medium can be proved also by the circumstance, that the substances so different in their chemical nature, as sodiumchlorid and cane sugar, evoke the same „closed“ development if used in isotonic solutions of a concentration coresponding to $\frac{n}{30}-\frac{n}{6}$ NaCl (my work 1914). $\frac{n}{30}$ KCl solution is too poisonons to allow for any development of the eggs whatever, therefore KCl solutions cannot evoke the „closed“ development.

I did not find obviously any „closed“ development in the distilled water. In the latter, though it is evidently injurious for the adult Daphnias, which die often in few hours if placed in it, the eggs go quite normally through all stages of the embryonal development.

I found in my experimental cultures the „closed“ development also in some other Cladocera species e. g. *Simocephalus vetulus, Ceriodaphnia laticaudata, Pleuroxus aduncus.* The closed development of their eggs was observed in $\frac{n}{20}-\frac{n}{10}$ sodium chlorid- and isotonic van't Hoff's solutions. The eggs passed through their whole embryonal development within the eggmembrane and died as a rule the 4-th or 5-th day.

The results of the observations on the „closed“ development of Daphnia embryos can be summarised as follows:

1) The „closed“ development seems to be caused only by the rising of the osmotic pressure in the surrounding medium, the chemical composition of the latter having no influence upon this process. The necessary minimum of the osmotic pressure is about the pressure of the $\frac{n}{30}$ NaCl solution.

2) During the „closed“ development the embryo attained the stage coresponding to the endstage of the normal development before the leaving of the broodpouch.

3) The development of the outer and inner organs seems not

to differ from the development of the normal embryo, the resorbtion of the reserve materials seems however to be slower.

4) The growth of the embryo during the „closed" development goes on usually up to the limit of the expansibility of the eggmembrane; often this limit is not attained by the embryos growth.

5) Having attained the endstage of the development the closed embryo can live no longer than 2—3 days, in this period of time the general weakening of the embryo proceeds and ends with its death.

6) Embryos liberated from the eggmembrane before the attaining or in the time of the attaining of the endstage, can grow and continue to develop in the pond water as well as in the same solution in which they have developed hitherto, and even in the solution of a higher concentration.

7) The rate of growth after the liberating outof the eggmembrane depends in an inverse sense upon the concentration of the new medium. In pond water the liberated embryo in the course of 2 days attains the equal size of the normally developed young Daphnias.

8) In case the losed embryo is liberated later from the eggmembrane in the period of the decreasing vitality, its further growth and development becomes the more difficult the later the liberation takes place.

9) This disturbance of the growth and development takes place also in liberated embryos which exceptionally have passed through the „closed" development in a higher concentrated solution, in which usually the eggs are rendered incapable of development.

Theoretical.

The description of experiments finished, I think it to be useful to consider once more the phenomena of Daphnia embryonal development in connection both with the changes of inner condition in Daphnia embryo, and with the changes of outer conditions in the medium.

The maturing of the porthenogenetic eggs in ovaries and their laying into the broodpouch seem to be depending only on the inner condition, i. e. inner osmotic pressure, of the adult animal. After the eggs have passed into the broodpouch and assumed their

definite shape their further development depends no more on the inner condition of the mother individual but only on the physico-chemical nature of the medium, which, as it has been already proved, can easily penetrate into the broodpouch. The surrounding medium acts often even in the first moment, when the eggmass not yet formed appears in the broodpouch passing from the narrow aperture of the ovaries. The medium if poisonous or concentrated enough may impede in this very moment the formation of the eggs and change them into a shapeless mass. The chemical nature of this medium can act however only as long as the eggmembrane is not yet formed. After its formation the predominant, main factor influencing the further course of development is the osmotic pressure of the surrounding outer fluid. The cessation of the development in Daphnia eggs after the formation of the membrane in the salt-solutions remains in a strict connection with the changes of the osmotic pressure within the egg.

Data concerning the inner osmotic pressure in the Daphnides-eggs obtained by means of the volume-method are to be found in the work of St. Przyłęcki (1921). The measurements made by this author on the parthenogenetic eggs of *Simocephalus vetulus* and *Daphnia magna* bring the prove, that the inner pressure in the parthenogenetic egg, originally low, increases continually during the embryonal development, attaining by the end of the development in *Simocephalus* the triple, in *Daphnia* the quadruple value of the initial pressure. The author assumes that the osmotic pressure in the fluid contained in the broodpouch in normal condition is lower than the inner pressure in the earliest development stages of the investigated eggs. P. states that the inner osmotic pressure in the developing egg increases in consequence of the increasing amount of osmotically active substances contained in the egg. The increase of the inner pressure evokes: 1) the increase of the embryos volume, (the relation of the final to the initial volume in Simocephalus = 2:1, in Daphnia magna = 2·38:1), 2) after the exceeding of the outermost limit of the expansibility of the egg-membrane the bursting of that membrane, and 3) in consequence of the further increase of the pressure the bursting of the larval membrane. — The increase of the inner pressure, according to P. is caused only by the chemical changes of the substances contained in the egg from the beginning.

With these phenomena of the normal development we may compare the phenomena accompanying the development of eggs in the saltsolutions. (My experiments were made mostly on *Daphnia pulex*, but on the basis of observations as to the behaviour of eggs and embryos in the solutions one can assume that the height of the osmotic pressure in the eggs of this species is contained in the limits of the osmotic pressure in *Daphnia magna*- and *Simocephalus*-eggs).

The irregularly formed or abnormally small eggs, which in the moment of laying into the broodpouch are deprived of the amount of protoplasm necessary for the normal development, remain as a rule without development, even in normal conditions.

These cannot be taken into account. — The eggs of the normal size and shape, laid into the broodpouch in the presence of the saltsolutions, of the concentration $\frac{n}{15} - \frac{n}{10}$ for the most part do not develop either; often the eggmembrane is not formed at all. — As it has been already mentioned, in the initial period before the formation of the membrane the egg can be stopped in its development or even killed by the direct action of the solution ions, whereas the same ions in the same concentration do not produce any noxious effect on the eggs when protected by the eggmembrane.

Of how great an importance the eggmembrane is in the egg remaining in the saltsolution, may be proved by the fact, observed in my cultures, that the eggs of *Daphnia pulex* laid into the broodpouch in the presence of $\frac{n}{60}$ KCl do not develop, do not produce any membrane and desintegrate into a shapeless mass. (series 5.) whereas the eggs placed in the same solution after the formation of the membrane develop in a normal manner. The egg passes often apparently without any harm through this critical initial period, but a little later, after the formation of the membrane, its development ceases and in the egg the yolk and the ball of fat accumulates on one side and a colourless substance on the other; or the yolk is forced towards the centre of the egg and under the eggmembrane a fluid, probably identical with perivitelin, appears. In both cases no further development of the egg takes place. It was proved in amphibian eggs (Przyłęcki 1917) that the producing of perivitelin is always accompanied by the decrease of the

osmotic pressure both in the fecundated and infecundated eggs. It is very probable that in case of Daphnia eggs the producing of perivitelin is acompanied too by a decrease of inner pressure. The fall of vitality in such eggs may indicate this decrease. It was however not yet indirectly proved.

A certain percentage of the eggs laid in the solution pass successfully through the first period and after the formation of the membrane are exposed only to the osmotic pressure of the solution, as it has been explained in the foregoing chapter. The figures relating to the value of the osmotic pressure inside and outside the egg during the experiments with the solutions are now to be compared.

In NaCl- and other solutions in the concentration $= \frac{n}{15} - \frac{n}{10}$ $\Delta =$ $= 0{\cdot}24—0{\cdot}35^{\circ}$ C. In the initial development stage in the eggs of *Simocephalus* and *Daphnia magna*, which were investigated after the formation of the membrane, the inner osmotic pressure is defined by $\Delta = 0{\cdot}186—0{\cdot}245^{\circ}$ C (Przyłęcki). Wheras Δ can be taken as the measure of the osmotic pressure, the latter in the $\frac{n}{15} - \frac{n}{10}$ NaCl-solutions is higher than the osmotic pressure in the initial stage of the parthenogenetic egg, and the cessation of the development in the first stages could be explained by the withdrawing of the water trough the semipermeable eggmembrane. In the lower concentrations than the above mentioned e. g. in $\frac{n}{20}$ NaCl (Δ 0·1765° C), in which already from the beginning the inner pressure in the egg prevails over the pressure of the solution, further development is theoretically possible. A certain number of not developing eggs, even in that concentration, may be explained by the poisonous action of the ions before the formation of the membrane, to which the freshly laid eggs are resistent in different degrees. The eggs placed in the solution of that concentration after the formation of the membrane as a rule develop normally.

The eggs which attained in the solution the stage characterised by developing extremities and the membrane of which burst, pass through the further part of development normally as a rule and parallely with the eggs developing in the testculture. This was found in my experiments in the embryos of *Daphnia*, *Ceriodaphnia*, *Scaphole-*

beris and *Simocephalus*. The embryos in that stage show the inner osmotic pressure much higher than the pressure of the solution, in which the initial development of the eggs is possible. In this stage i. e. in the period when the Daphnia embryos are closed only in the larval membrane, they seem to be most resistent to the osmotic action of the saltsolution.

In the normal development the volume of the embryo in the final stage in the moment of leaving the broodpouch is at least thrice as great as the egg volume before bursting of the eggmembrane. On the contrary the closed development (described in the foregoing chapter) is not accompanied by the growth; the volume of the embryo remains from a certain moment unchanged. This phenomenon also may be explained by the relation of the outer to the inner osmotic pressure.

Białaszewicz found in the frogembryos that the growth during the extrauterin embryonal life proceeds chiefly by absorbtion of water from the surrounding medium, and only to a slight extent by the absorbtion of nutritious substances from the jelly surrounding the egg. In *Daphnia* the latter does not exist. All reserve materials are contained inside of the egg. Whereas no nutritious substances are taken from the fluid of the broodpouch (as stated in my work from 1914) the absorbtion of water from outside remains as the only factor causing growth. The water is absorbed by the egg owing to the preponderance of the inner pressure evoked by the forming of the osmotically active substances inside of the egg. If we assume that the development proceeds in the distilled water, the preponderance of the inner over the outer pressure in the stage when the eggmembrane normally bursts should be $\Delta = 0{\cdot}75^{\circ}$ C. In the pondwater this preponderance should be a little less; (the osmotic pressure of the pondwater used in my experiments, the chemical composition of which is given in the first chapter is about $\Delta = 0{\cdot}02^{\circ}$ C). In the saltsolution the higher the concentration the less is the preponderance of the inner pressure in the egg over the outer pressure of the medium. In $\frac{n}{20}$ NaCl ($\Delta = 0{\cdot}176^{\circ}$ C) the closed development occurs nearly as often as the normal one. The pressure of this solution can thus be still equaled and surpassed by the inner osmotic pressure of the developing egg. The maximum for such a surpassable outer pressure seems to be between

$\frac{n}{20}$ — $\frac{n}{15}$ NaCl. — If however the eggmembrane does not burst in the proper time, the embryo remains closed till its death which follows as a rule after attaining of the final embryonal stage. One could admit that in the closed embryo no new osmotically active substances are produced which could raise its inner pressure. In a closed embryo the amount of the non absorbed yolk-materials is greater than in a normal embryo in the same stage. A causal connection between the absorbtion of the reserve materials inside of the embryo and the rising of its inner pressure seems thus to be evident.

Although the assimilation in the closed embryo seems to be checked to a certain extent, the process of development proceeds. — This state cannot be compared with the state of latent life characteristic of the wintereggs of Cladocera. If not interrupted this state ends with death of the embryo. It is very likely that the poisoning by the products of the change of matter plays the main rôle in that case. This state however when interrupted early enough passes into the state of normal life and the rapid growth of the embryo connected with the abundand absorbtion of water proves the existence of preponderance of the inner pressure in the embryo in that stage over the pressure of the solution. — The fact that the later the closed embryo is liberated from the eggmembrane, the slower and weaker does proceed its further growth, might prove that parallely to the weakening of the embryo decreases its inner osmotic pressure.

The larval membrane plays no important rôle during the closed development. The closed Daphnia embryos being artificially liberated from the eggmembrane after attaining of the endstage, the larval membrane remains on them for various length of time, depending upon the concentration of the medium, into which the embryos had been transferred after the liberation. In the pure pond water the larval membrane bursts after 2—3 hours, in $\frac{n}{20}$ NaCl it remains for several hours, in $\frac{n}{10}$ NaCl it ramains up to 20 hours. The time necessary to its bursting is thus dependent on the more or less intensive absorbtion of water by the embryo. As a checking factor for the growth of the embryo the larval membrane

is of a minimal importance on account of its considerable expansibility and very slight coherence.

The closed development in Daphnia embryos may be regarded as a proof of lack of accomodation in the parthenogenetic egg to the solution. The Fritzsche's investigations proved that the adult Daphnias have a considerable ability of accomodation to the solutions due to production in a relatively short time of the preponderance of the inner pressure over the pressure of the solution into which they had been transferred. — If the egg of Daphnia, placed into the saltsolution, could accomodate to the new conditions, it should produce a due increase of inner osmotic pressure, higher than in normal conditions, which surpassing the outer pressure, could cause the bursting of the eggmembrane.

Here may be the place for some remarks concerning the question of accomodation in Daphnia to the saltsolutions. — This question was not the special object of my experiments. Some observations however of mine concerning the conditions of the eggdevelopment may contribute to a certain degree to the explanation of this question.

The accomondation to the saltsolution in Daphnia has been thoroughly investigated by several authors, among others by P. Bert, E. Warren, A. Dernoschek, E. Hirsch, H. Fritsche. — They have come to conclusions differing from each other. In the works of three latter authors we find exhaustive lists and discussion of the literature concerning that subject and the question of accomodation in general. Therefore I shall cite here only the principal results of the experiments made in genus *Daphnia*, obtained by the above authors, and not take into consideration the experiments made on other animal forms.

P. Bert comes to the conclusion that in Daphnia is no accomodation in individual but in the species and appear in the second generation: „ily'a ainsi une acclimatisation non dans l'individu, mais dans l'espece". E. Warren comes also to the negative conclusion regarding the first generation, stating that the animals, which had passed a certain preparative time in a weak solution, are not at all more resistent to the stronger solutions, than the animals which had been taken directly from the fresh water, and that the accomodation in Daphnia to the NaCl-solutions does not surpass 0·55%

solution $\left(\frac{n}{10}-\frac{n}{11}\right)$. From one experiment of his however may result, that the second generation is more resistent to the action of the solution than the first generation. E. Hirsch finds in Daphnia a lack of accomodation to the NaCl-solutions and to the solutions of other sodium-, potassium- and calcium salts, with the sole exception of $CaCl_2$. H. Fritzsche on the strength of his measurements of the inner osmotic pressure in Daphnia states that the accomodation can appear in the first generation already and that it depends in the first place on the ability in the investigated individual to produce the preponderance of the inner pressure over the pressure of the solution. F. found the accomodation to the seawater solutions isotonic with $\frac{n}{8}$ NaCl in adult Daphnias in the individuals cultivated in the solutions (the concentration of which gradually increased by evaporation) as well as in individuals transferred into it directly from the pond water. The individuals which had produced the preponderance of the inner pressure die after a certain time, the author supposes however that they might be kept alive, if the increasing of the outer pressure were slower and more gradual. A. Dernoschek comes to a positive conclusion as refers to the accomodating power of *Daphnia magna* to the seawater solutions; the results of his experiments however are hardly to be compared with the results of the afor mentioned authors, wheras D. deals only with high concentrations killing the Daphnias in a short time and his cultures did not aim at producing a new generation in the solution.

As regards the accomodation to the saltsolutions in adult forms, two separate questions may be thus distinguished in experiments of above authors: 1) the accomodation in an individual, 2) the accomodation in the species which may appear at first in the subsequent generation. My observations were directed only to the embryonal period of life. As regards the phenomena of an accomodation in the eggs, they might appear without mediation of the adult forms only in the first generation. One could speak of an accomodation in eggs: 1) if the eggs, which had passed their first initial period in the solution, showed a greater resistibility to the further action of this solution than the eggs which in a later stage of development had been placed in it, or 2) if the eggs which

had passed a certain time in a weaker solution, transferred into like solution of a stronger concentration bore its action better than the eggs transferred directly from the pure water into this stronger solution. — I found no confirmation of these cases in my experiments: as regards the first point the unprepared eggs could bear even a stronger solution than the eggs which had passed their first period in the solution, as they were not injured by the salt ions during this first, most sensible period of life; — as regards the second point, no accomodation of such a kind was to be noticed in my experiments. That the phenomenon of the „closed" development may also serve as a proof of lack of accomodation in the eggs, it was already emphasised. As to the accomodation in the second generation it can be found in a certain though slight degree. From the eggs freshly laid into the broodpouch those of the animals bred in the solution seem to have no more resistance to this solution than the eggs of the animals bred in the pond water and afterwards transferred into the solution before the laying of the eggs. The sensibility to the direct action of the salt ions seem to remain unchanged. But as to the later course of development, in the period following the formation of the eggmembrane, the eggs laid by the animals bred in solutions, show very slight percentage of „closed" development cases, whereas among the eggs laid by animals transferred from pond water to solution, the percentage of „closed" development cases is predominant. — This applies to the concentrations $\frac{n}{24}-\frac{n}{15}$ NaCl and isotonic van't Hoff's solutions. In stronger solutions $\left(\frac{n}{12}\text{ NaCl}\right)$ the eggs, even when laid by the animals bred in solution, develop closed in the membrane. — On the strength of the above described facts one could admit in Daphnia a certain though limited accomodation power to the saltsolutions, noticeble also in the embryonal life but in the second generation.

To the statement that the eggs of the second generation show a certain accomodation to the solution in comparison with the eggs of the preceding generation it may be objected that this two generations are not equal in regard to their vitality. The animals of the second generation ought to show a higher resistance power as they belong to the first brood, and the animals of the preceding generation often belonged to a late brood of this generation, and it

is proved by the experiments of Woltereck and Papanicolau that the later brood the eggs belong to the weaker is their vitality. — But the testcultures in my experiments showed, thad the eggs of the same material when living in the pond water developed quite normally thus they did not belong to the late and weakened broods as well as the eggs placed in solutions, and that all abnormalities in the development which appeared in the saltsolutions cultures were due only to the action of these solutions.

In my experimental cultures mostly sodium chlorid-, Ringer- and van't Hoff's solutions were used. As it had been mentioned already in the first chapter, I could not prove any indubitable, distinct difference in the action of these three kinds of solutions upon the eggs of Daphnids, either in the younger or in the older ones. J. Loeb's experiments on *Fundulus* and on the sea urchin eggs and W. Ostwald experiments on *Gammarus pulex* proved that the adding of a small quantity of KCl- and $CaCl_2$ solutions to the isotonic NaCl-solution, strictly defined by the distoxication-coefficient (Entgiftungs-koeffizient) diminisches to a great extent its poisonous properties; this poisonous action is yet more diminished by the adding of magnesium salts solutions ($MgCl_2$ and $Mg\ SO_4$) to the mixture of those three solutions. The composition of these 3 resp. 5 salts if in a proper proportion, as contained in Ringer- and van't Hoff's solution, causes the weakening of the poisonous properties which each of those salts should manifest if used alone in the experiment. — But both authors, Loeb and Ostwald, used mostly in their experiments the solutions of a strong concentration, killing the animals in a comparatively short space of time; there was no question of keeping the individuals alive for a longer time and of making observations upon their reproduction. In those higher concentrations the difference between the action of the pure NaCl-solutions and the compound solutions was conspicuous. E. g. in Ostwald's experiments the average lenght of life of the adult *Gammarus pulex* in 1·53% NaCl solution was 243 minutes, in the isotonic triple solution of $NaCl + KCl + CaCl_2$ (in the proportion 100:2·1:1, much approached to Ringer solution) about 13 hours, in isotonic van't Hoff's solution about 96 hours. The 1·53% NaCl solution was the weakest among those used by Ostwald and the scale of concentration reached 4·38% NaCl. In my experiments upon the vitality of the eggs to which much lower concentrations were used (0·15%—

0·6%), in order to render the reproduction possible, the counteraction of the same salts was difficult to be found.

Summary.

1. Sodium chlorid-, Ringer- and van't Hoff's solutions restrain the passing of the parthenogenetic eggs from the ovarium into the broodpouch in *Daphnia pulex* and some other Cladocera species. This restraining action can be either total or partial. It beginns to appear in the concentration $= \frac{n}{30}$ NaCl and in concentrations above $\frac{n}{10}$ NaCl the restraining is total.

2. This restraining action upon the passing of the eggs into the broodpouch appears in individuals living in solutions before the weakening of their other vital functions. Nevertheless it may be assumed that the chemical and osmotic properties of the solution exercise an indirect influence upon the first above mentioned function by the enfeebling of the egg-producing female (by reducing of her inner osmotic pressure).

3. Sodium chlorid-, Ringer- and van't Hoff's solutions exercise a restraining influence upon the development of the parthenogenetic eggs. This influence can be noticed in the eggs brought artificially outof the broodpouch as well as in the eggs contained in it and brought into the contact with the solution by placing of the adult animal into the latter. This influence beginns to appear in $\frac{n}{30}$NaCl- and isotonic Ringer- and van't Hoff's solutions and increases parallely to the concentration.

4. The eggs after the formation of the eggmembrane can develop in NaCl solutions up to the concentration $\frac{n}{10} - \frac{n}{6}$. Their development power depends upon the stage in the moment of placing them in the solution. This power increases with the age attaining its maximum after the normal bursting of the eggmembrane.

5. Seperately, as most sensitive, must be regarded the first, (very short), membraneless period in the life of egg when the latter is exposed to the direct chemical action of the salt ions. Therefore the eggs which pass into the broodpouch during the abode of adult

animal in the solution are not able to develop in the concentration above $\frac{n}{12}$ NaCl.

6. The vital condition of the adult individual has no influence on the development of the eggs remaining in its broodpouch; this development depends solely upon the physical and chemical properties of the experimental solution.

7. The eggs of the succeeding broods of the same female living in the solution show less and less resistibility to the action of this solution. This lessening of the resistibility seems to corespond with the lessening of the vitality and of the development power in the eggs of succeeding broods in natural conditions.

8. The sodium chlorid-, Ringer- and van't Hoff's solutions evoke a „closed" development in eggs when placed in solution after the formation of the membrane. Development of the organs is relatively normal but the growth is entirely stopped and the eggmembrane does not burst. This phenomenon is caused only by the increase of the osmotic pressure in the outer medium, the chemical nature of the solution being here of no importance. Such a development ends with the death of the embryo, the process can however be artificially interrupted and can change into a normal development.

9. Among the eggs of the same brood living in the solution there are considerable differences as to the power of development. They seem to be caused by slight oscillations in the vitality among the eggs, which being not perceptible in normal conditions, come to light under the influence of dissolved salts.

10. The period of time necessary for attaining the final embryonal stage is equally long in the eggs passing through the normal development in the pond water, through the normal development in the solution and through the closed development in the solution (in the 2 latter cases up to the concentration $= \frac{n}{15}$ NaCl).

11. The animals, which passed through their total development in the solution, attain their maturity in the same space of time, sometimes even earlier, than the animals from the normal culture. In the third generation bred in the solution both, the period of embryonal development and of the attaining of maturity is as long

as in the second generation. This applies to the solutions of a concentration weaker than $\frac{n}{15}$ NaCl.

12. The number of eggs in single broods produced by the females bred in the solution is not smaller than the number of eggs produced in parallel natural cultures; this may prove that given equal feeding conditions the assimilation of the food is as intensive in the solution as in the freshwater.

13. The rate of reproduction, (spaces of time between the moments of saparate broods passing into the broodpouch) are as long in the above mentioned solutions as in the normal cultures, unless the female is eminently weakened by the action of the solution.

14. The parthenogenetic eggs, passing through their development in the solution, do not show as a rule a greater resistibility to its action than the eggs transferred in a certain stage of development from the freshwater to the solution. A certain acclimatisation is to be noticed but in the second generation, the eggs of which are able to pass through their normal development in a concentration slightly higher than the eggs of the first generation. — This applies to the weaker solutions $\left(\text{upto } \frac{n}{20} \text{ NaCl}\right)$.

The present experiments were carried out in the Zoological Institute of the Jagellonian University Cracow. I take this opportunity to express to the Director of the Institute Prof. Dr Michael Siedlecki my best thanks for the interest he has taken in my work and his aid in obtaining the necessary literature.

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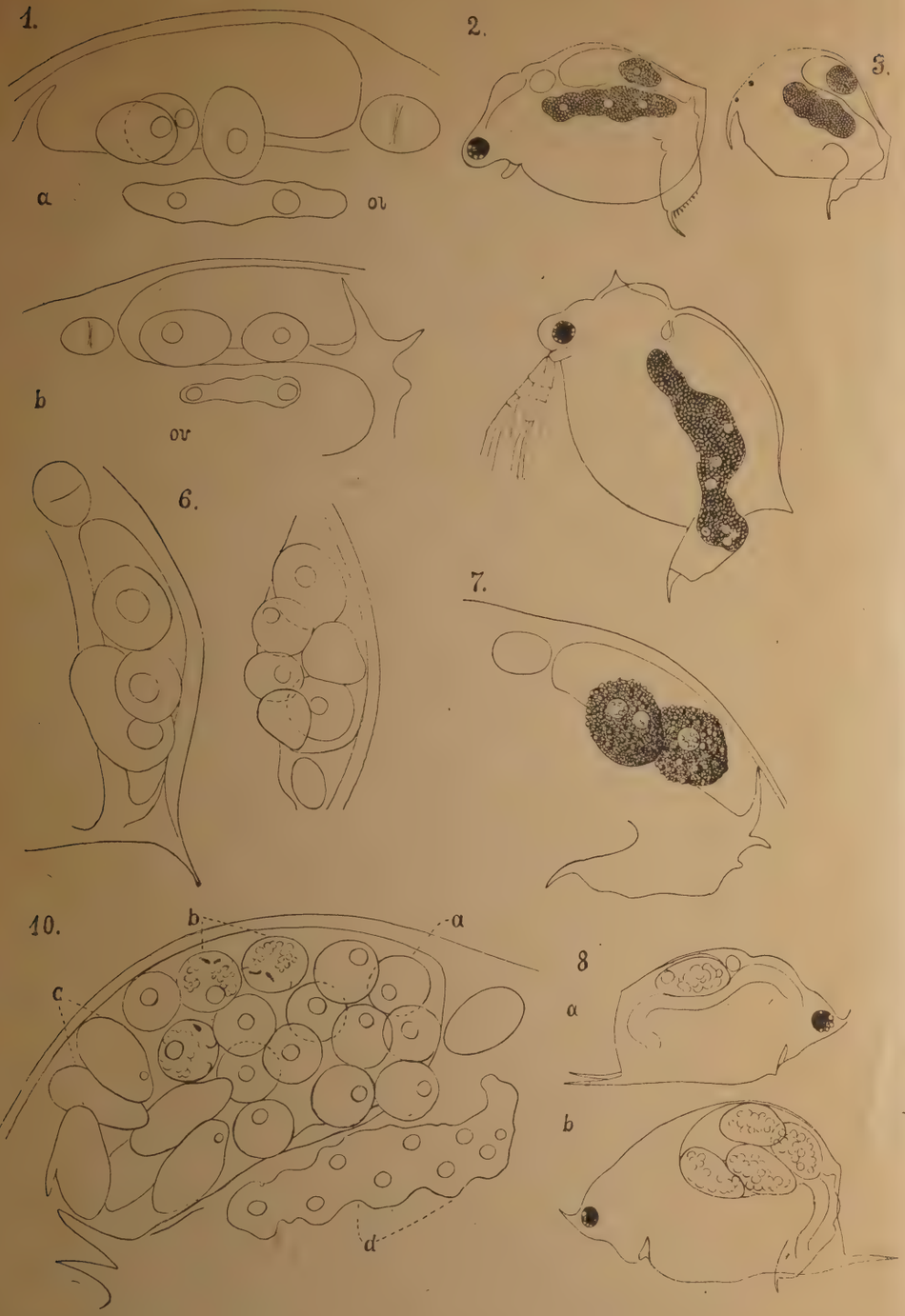
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Explanation of the plate 8.

Fig. 1. Partial passing of the parthenogenetic eggs into the broodpouch in *Daphnia pulex* remaining in van't Hoff's solution. a) in solution isotonic with $\frac{n}{10}$ NaCl, b) isotonic $\frac{n}{20}$ NaCl, c) ovaries in which the passing of the eggs was checked.

Fig. 2. *Ceriodaphnia*, partial passing of the eggs into the broodpouch in $\frac{n}{15}$ NaCl solution.

Fig. 3. *Pleuroxus*, partial passing of the eggs into the broodpouch in $\frac{n}{12}$ NaCl solution.

Fig. 4. *Daphnia pulex*, in $\frac{n}{60}$ KCl solution. The maturing parthenogenetic eggs passed into the moult of postabdomen instead of passing into the broodpouch.

Fig. 5. *Ceriodaphnia*, in $\frac{n}{15}$ NaCl solution. The maturing parthenogenetic eggs passed into postabdomen cavity instead of passing into the broodpouch.

Fig. 6. Parthenogenetic eggs of irregular size and shape laid in *Daphnia pulex* in $\frac{n}{20}$ NaCl.

Fig. 7. Parthenogenetic eggs laid in *Daphnia pulex* in van't Hoff's solution isotonic with $\frac{n}{10}$ NaCl; the eggmembrane is not formed.

Fig. 8. *Scapholeberis mucronata*, a) bred in pond water, b) bred in $\frac{n}{30}$ NaCl-solution; 30 hours from the moment of egg laying into the broodpouch; in both animals in the same space of time the same stage was attained.

Fig. 9. Parthenogenetic eggs of three subsequent broods in *Daphnia pulex* laid in $\frac{n}{30}$ NaCl- (comparing of their size). a) first brood, in ovaries maturing eggs of the second brood, b) second brood, in both eggs development impeded by the action of the solution.

Fig. 10. *Daphnia pulex*, differences in development power in eggs laid into broodpouch in van't Hoff's solution isotonic with $\frac{n}{15}$ NaCl; a) eggs stopped in development, b) embryos in the stage of paired eye („closed" development), c) embryos in the same stage — normal development, d) maturing eggs of the second brood in ovaries.

Fig. 11. „Closed" development of *Daphnia pulex*- embryo in van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl. c — heart, o — ball of fat, v — yolk materials.

Fig. 12. Growth of the parthenogenetic eggs in *Daphnia pulex* during the „closed" development in van't Hoff's solution isotonic wifh $\frac{n}{20}$ NaCl. a) 18 hours from the moment of passing into the broodpouch, b) stage in which normally

burst the eggmembrane, c) „closed“ development, 70 hours from the moment of passing into the broodpouch, d) the same embryos (as in c) just after the perforation of the eggmembrane (closed in larval membrane).

Fig. 13. The same embryos (as in Fig. 12. a—d) 4 hours after perforating of the eggmembranes — immedialety before the perforation transferred — a) into van't Hoff's solution isotonic with $\frac{n}{10}$ NaCl, b) into van't Hoff'ssolution isotonic with $\frac{n}{20}$ NaCl, c) into pond water. a—b) closed in larval membranes, c) larva membrane burst.

Fig. 14. Different rate of growth in *Daphnia pulex*-embryos after the artificial interrupting of „closed“ development in van't Hoff's solution isotonic with $\frac{n}{20}$ NaCl, all embryos of the same age (122 hours from the moment when the eggs were laid into the broodpouch) — a) liberated from the membrane after 118 hours, b) liberated after 94 hours, c) liberated after 70 hours.

Fig. 15. Equation in growth between the *Daphnia pulex* embryos normally developing and those artificially liberated from the „closed“ development. a) embryos the same as in Fig. 14 c, after further 48 hours abode in pond water, b) embryos of the same age normally developing from the beginning.

Fig. 16. Growth of *Daphnia pulex* embryos after the artificial interruption of „closed“ development in van't Hoff's solution isotonic with $\frac{n}{12}$ NaCl. a) 60 hours of „closed“ development immediately before the perforation, b) 6 hours after the perforation of the eggmembrane, c) 18 hours later; on that stage growth and development stopped.

All figures were drawn by means of Abbe-apparatus.

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